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The *in vivo* Activity of Several Systemic Insecticides against Cattle Grubs in South Dakota[1]

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ABSTRACT

Data are presented on the activity of several successful and unsuccessful materials for systemic control of cattle grubs, *Hypoderma lineatum* (De Vill.) and *H. bovis* (L.). Ronnel, applied as drench or bolus at approximately 110 mg./kg., proved highly effective (99% or better) except where infestation levels averaged less than one grub per head. Dimethoate, oral as well as intramuscular at 15 mg./kg., was highly effective (94%) but was accompanied by depression (as much as 56%) of cholinesterase activity coupled with at least initial weight gain deceleration. Dowco 109 (*O*-(4-*tert*-butyl-2-chlorophenyl) *O*-methyl methylphosphoramidothioate) when sprayed at a concentration of 0.75% proved highly effective (100%) and was accompanied by no detectable cholinesterase depression, but when sprayed at 0.5% over the entire body or on the backs of cattle alone, this material failed or gave marginal control (27%–76%). Bayer 21/199 (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate, as wettable powder or as emulsion, was sprayed at 0.5% and at 0.25% under conditions of complete or partial coverage of cattle. The results with this material (at 0.5%) show considerable irregularity, ranging from excellent (91% to 97%) to poor (under 65%) but not directly correlated with formulation or degree of coverage. The tests at 0.25% concentration failed (43% control or less). Nicarbazin (complex of 4,4′-dinitrocarbanilide and 2-hydroxy-4,6-dimethyl pyrimidine), provided as a multiple oral dosage of 25 mg./kg., was unsuccessful (26%), but when administered as single oral dosages at 125 mg./kg. was successful though poor (65%), and at 250 mg./kg. was moderately successful (86%). Six other materials, each of which had shown promise in *in vitro* tests, were unsuccessful (27% control or less); these materials are: (I) 9-amino-6-chloro-2-methoxyacridine; (II) 4,4′ dimethylbenzhydryl amine HCl; (III) di-n-butyl tin dilaurate; (IV) 1-phenyl-3-(5-nitro-2-thiazolyl) urea; (V) 2-(p-nitrobenzamido)-5-nitrothiazole; and Monacrin®, 9-aminoacridine HCl.

The data presented on gains in weight do not provide evidence of a relationship between grub control and weight-gaining ability of calves maintained under dry-lot conditions. In general, the degree of grub control obtained appeared better in experiments involving heavily infested cattle than in lightly infested animals.

In 1953 a search was initiated at the South Dakota Agricultural Experiment Station for compounds of potential value as systemic agents for the control of cattle grubs, *Hypoderma lineatum* (De Vill.) and *H. bovis* (L.), utilizing an *in vitro* technique patterned after that of Barrett & Wells (1948). Promising materials were challenged further by various treatments *in vivo* of grub-infested calves. Results of some of these tests were reported by Rogoff & Kohler (1956), Rogoff *et al.* (1957), and Kohler *et al.* (1959).

Following the announcement of ronnel by Lindquist (1956), this material was incorporated in the *in vivo* studies that were already underway. As other systemic agents became available they, too, were included in these tests. Related studies on low-level feeding of ronnel were reported by Rogoff & Kohler (1959) and by Kohler, *et al.* (1959). This paper summarizes studies conducted *in vivo* on materials derived from the *in vitro* tests and from commercial sources.

MATERIALS AND METHODS.—The chemicals utilized in this study are described in detail in table 1. Materials I through V, Monacrin, and nicarbazin had shown earlier promise in the *in vitro* studies at this laboratory, and, with the exception of III, have not been tested elsewhere against *Hypoderma* as far as is known. Compound III, di-n-butyl tin dilaurate, is one of the constituents of Hypolin, a British proprietary based on phenothiazine and used successfully against cattle grubs by Worden (1953), but without success by Kohler & Rogoff (1959). Cobbett *et al.* (1957) tested di-n-butyl tin dilaurate in cattle without success. These materials were locally formulated from technical samples. The other four compounds have been studied in varying degrees throughout the world.

Cattle involved in these tests originated in areas of South Dakota where a high incidence of cattle grubs would normally be expected. Some of the animals were trucked to feedlots maintained at the Agricultural Experiment Station in Brookings, South Dakota; some were held at substations of the Agricultural Experiment Sta-

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[2] Entomologist, Associate Animal Husbandman, and Assistant Animal Husbandman, respectively, in the South Dakota Agricultural Experiment Station. Some of the data included herein were submitted by the second author to the Graduate Faculty of the University of Minnesota in partial fulfillment of the requirements for the Ph.D. degree.

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Table 1.—Chemical composition and source of materials used in this investigation.

MATERIAL	SYNONYMS AND COMPOSITION	SOURCE
I	9-amino-6-chloro-2-methoxyacridine	Squibb Institute
II	4,4′ dimethylbenzhydrylamine HCl	Merck and Co.
III	di-n-butyl tin dilaurate	Dr. Salsbury's Laboratories
IV	1-phenyl-3-(5-nitro-2-thiazolyl)urea	Merck and Co.
V	2-(p-nitrobenzamido)-5-nitrothiazole	Merck and Co.
Monacrin®	9-aminoacridine, HCl (1954)	Squibb Institute
Monacrin®	9-aminoacridine, HCl (1955)	Bayer Pharma, Sydney
Nicarbazin	complex of: 4,4′-dinitrocarbanilide and 2-hydroxy-4,6-dimethyl pyrimidine	Merck and Co.
Ronnel	Dow ET-57; Trolene®; *O,O,*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate	Dow Chemical Co.
Bayer 21/199	Co-Ral®; *O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate	Chemagro Corporation
Dimethoate	Am. Cyanamid 12,880; *O,O*-dimethyl S-(*N*-methylcarbamoylmethyl) phosphorodithioate	American Cyanamid Co.
Dowco 109	*O*-(4-*tert*-butyl-2-chlorophenyl) *O*-methyl methylphosphoramidothioate	Dow Chemical Co.

tion in central and in western South Dakota; and some animals were in commercial herds operated by cooperating ranchers in central South Dakota.

Tests conducted at Brookings were on calves lotted by weight and sex (except where specifically noted) and held in adjacent sheds and yards. Hay, grain, and water were provided in a comparable manner for each lot. Brome hay was offered free-choice. A mixture of equal parts of oats and of corn was offered to each lot on the basis of 4 pounds per calf per day. All of these calves were individually weighed at monthly intervals. Details of the substation and cooperator tests are given with the descriptions of the individual tests.

Modes of administration of the candidate chemicals to cattle were by oral drench, oral bolus or oblet, subcutaneous injection, spray, or wash applied to the back. Materials were evaluated either by periodic palpation or by extraction of the encysted grubs. Data derived by palpation are so noted in the tables. Extracted specimens were identified to species. Where possible, individual weights of the experimental animals were periodically determined. Studies on erythrocyte cholinesterase activity utilized the method of Michel (1949).

RESULTS.—Tests conducted in the 1954–55 grub season were entirely on calves held in drylot yards in Brookings. The materials used were chosen from those showing promise in the *in vitro* tests of the previous year (using oesophageal stage *H. lineatum* larvae), and were administered on a single-dose basis. The data are shown in table 2. The infestation of all calves in this series ranged from 16 to 192 grubs per head. It is apparent that under the conditions of these tests no promise was indicated by compounds I and II, nor by Monacrin.

The 1955–56 tests (table 3) were conducted in a manner comparable to those of the previous year except that the dosages were repeated at four monthly intervals. Of the compounds used the previous year, only Monacrin was retested. The infestation range again was high, with a spread from 21 to 98 grubs per head in all calves under test. No significant degree of control was observed for compounds III, IV, V, nicarbazin, or Monacrin. The differences observed in gain in weight also were not significant.

In the third year (1956–57) of this series of tests, emphasis was shifted to the newly-available ronnel, though a few other compounds were also included. Yard tests at Brookings were conducted in a manner similar to those of the previous 2 years, and are described in table 4. Of the materials used, nicarbazin was the only one repeated from the year before. It was used at 5 and at 10 times the initial dosage of the earlier test in order to evaluate it at a level at least as high as that chosen for the work with ronnel. The data in table 4 indicate poor to moderate reductions (65% and 86%) in the four animals under test. Ronnel (unformulated technical material) was adminis-

Table 2.—Effectiveness of several candidate compounds administered once to calves in November and December, 1954. Brookings, S. Dak.

MATERIAL	PROCEDURE AND DOSAGE	NO. OF CALVES	GRUBS/CALF		PER CENT CONTROL
			RANGE	AVERAGE	
I	sub-cut.; 10 mg./kg.	3	85–192	137.6	0
I	drench; 10 mg./kg.	4	18–114	47.2	25
II	drench; 10 mg./kg.	4	37–183	82.5	0
Monacrin	drench; 20 mg./kg.	4	35– 77	45.8	27
Untreated	—	4	16–120	63.0	—

Table 3.—Effectiveness and weight gain following administration to calves of several candidate compounds as four monthly drenches starting in December, 1955. Brookings, S. Dak.

MATERIAL[a]	TREATMENT		GRUBS/CALF		PER CENT CONTROL	AVERAGE DAILY GAIN (LB.)[b]
	GRAMS /MONTH	MG./KG. (INITIAL)	RANGE	AVERAGE		
III	2	10	21–73	55.6	0	1.64
IV	5	25	26–76	44.0	20	1.93
V	5	25	31–74	47.0	15	1.74
Nicarbazin	5	25	34–48	41.0	26	1.61
Monacrin	5	25	42–98	69.3	0	1.55
Untreated	—	—	41–80	55.3	—	1.89

[a] Three calves used in each category.
[b] Not significant at 5% level. Test ran for 135 days.

Table 4.—Comparative effectiveness and weight gain following the single administration to calves of several compounds in December, 1956. Brookings, S. Dak.

MATERIAL	PROCEDURE AND DOSAGE	NO. OF CALVES	GRUBS/CALF[a]		PER CENT CONTROL	AVERAGE DAILY GAIN (LB.)[b]
			RANGE	AVERAGE		
Nicarbazin	oral (capsule)—125 mg./kg.	2	24– 31	27.5	65	1.38
	oral (capsule)—250 mg./kg.	2	10– 12	11.0	86	1.07
Ronnel	oral (capsule)—115 mg./kg.	8	0– 1	0.1	99	1.41
Bayer 21/199	back (wash)—0.75%[c]	4	4– 23	11.3	86	1.61
Untreated	—	8	19–147	78.3	—	1.48

[a] Based on palpation Jan. 25, and extractions Feb. 23 to 25 and Mar. 25.
[b] Not significant at 5% level. Test ran for 112 days.
[c] Applied 1½ qts. suspension. Equivalent to 100 mg./kg.

Table 5.—Comparative effectiveness of ronnel drenches in calves, yearlings, and cows administered at 110 mg./kg. in November, 1956 to two cooperators' herds in Central South Dakota.

Criteria	Ranch A		Ranch B			
	March 21[a]		March 2[a]		March 30[a]	
	Drench	Untreated	Drench	Untreated	Drench	Untreated
Cows and yearlings						
No. of cattle	30	33	21	22	21	22
Infestation (%)	13	27	10	18	14	18
Grubs/head (range)	0–4	0–12	0–1	0–8	0–6	0–12
Average grubs/head	0.3	1.0	0.1	0.7	0.4	0.9
Reduction (%)	33	—	86	—	66	—
Calves						
No. of cattle			18	22	18	22
Infestation (%)			0	68	0	77
Grubs/head (range)			0	0–12	0	0–15
Average grubs/head			0	4.0	0	5.7
Reduction (%)			100	—	100	—

[a] Palpation date, 1957.

tered in capsules using a balling gun. The results are spectacular, only one grub having appeared among eight calves, compared with an average of over 78 grubs per head in the eight control animals. Other calves in this same series were allowed long-term access to ronnel at low levels of ingestion, data already reported by Kohler *et al.* (1959) and by Rogoff & Kohler (1959). Bayer 21/199 was applied as a wash to a small number of calves. The application technique consisted of pouring the 0.75% suspension on the back of each calf while simultaneously scrubbing with a brush. The application technique was not ideal. The results, while interesting, are not comparable with those obtained with ronnel.

The yard tests in 1956–57 were supplemented by tests on cooperators' herds in central South Dakota. Animals were ear-tagged as they came through the chutes, individual weight was estimated, and every other animal was drenched with an amount of slurry made from a wettable powder containing 25% of the active ingredient to provide a dosage of 110 mg. of ronnel per kg. of the host. The results of these tests are shown in table 5. The low infestation in untreated cows and yearlings should be considered in evaluating the results of these tests. It is apparent, however, that excellent control was achieved among the calves, with relatively indifferent results among the older animals.

Tests conducted during the fourth year (1957–58) were aimed at further evaluations of ronnel and Bayer 21/199, as well as the new compounds, dimethoate and Dowco 109. The yard tests at Brookings included studies on low-level, free-choice accessibility of ronnel in salt (already reported by Rogoff *et al.* 1959) and on single administration of dimethoate or Dowco 109 (table 6). These studies also included effects on erythrocyte cholinesterase and on gains in weight as well as on infestations. The data indicate a high degree of control by oral and intramuscular administration of dimethoate at 15 mg./kg. and by the total coverage, high-pressure spray of 0.75% Dowco 109 suspension. The cholinesterase studies indicate considerably reduced activity in the red cells of the dimethoate-treated calves, but not in those exposed to Dowco 109. The weight-gain studies over the 111 day period do not indicate any large differences among treatments, although

Table 6.—Infestation, erythrocyte cholinesterase, and weight gain following the single administration of dimethoate or Dowco 109 to calves in December, 1957. Brookings, S. Dak.

Criteria	Treatment			
	Dimethoate "Oblet" (15 Mg./Kg.)	Dimethoate Intramuscular (15 Mg./Kg.)	Dowco 109 Spray[a] (0.75%)	Untreated
Total calves	4	8	8	8
Infestation				
Calves infested	2	1	0	6
Grubs/calf (range)	0–1	0–3	0	0–36
Average grubs/calf	0.8	0.8	0	13.1
Reduction (%)	94	94	100	—
Erythrocyte cholinesterase				
	January 17, 1958 (25th day)			
Range[b]	0.16–0.30	0.14–0.22	0.22–0.52	0.30–0.49
Average[b]	0.22	0.17	0.33	0.39
Activity (% initial)	55	44	87	83
	February 14, 1958 (53rd day)			
Range[b]	0.28–0.41	0.21–0.40	0.31–0.55	0.32–0.55
Average[b]	0.35	0.28	0.40	0.45
Activity (% initial)	88	72	108	96
Average weight gain/calf				
Initial weight (lb.)	412	410	399	398
Daily gain (lb.)[c]	1.55	1.61	1.73	1.62
Per cent of initial weight	42	44	48	45

[a] More than 1 gal./calf, 450 p.s.i.
[b] ΔpH units/hour.
[c] Not significant at 5% level. Test ran 111 days.

the data favor the Dowco 109 treatment. In the first month following treatment, however, the calves treated with dimethoate gained less (0.79 pound per day for the oblet and 0.84 pound per day for the intramuscular treatment) than the eight Dowco 109-treated calves (1.38 pounds per day) or the controls (1.30 pounds per day). Throughout the rest of the study period the rate of gain of the dimethoate-treated animals was higher than that of the others so that by the end of the period they had essentially caught up with their herd-mates.

The Bayer 21/199 field tests in 1957–58 were applied in two sharply contrasting degrees of thoroughness. The first of these (table 7) involved calves at the Cottonwood Substation. These animals were carefully sprayed (pressures 250 to 400 p.s.i.) in well-arranged holding facilities. The degree of control achieved (84% to 87%) was moderate, but not excellent. The second field test (table 8) included yearlings and cows as well as calves of a group of commercial herds that had run together during the spring and summer. Application was made with the same equipment and by the same operators as at the Cottonwood Substation. The calves were reasonably well

Table 7.—Effectiveness of Bayer 21/199 (0.5% suspension) sprayed on calves November 20, 1957. Cottonwood, S. Dak.

Criteria	Counts by Palpation 1958	
	February 6 (Treated/Control)	March 6 (Treated/Control)
Total calves	43/34	43/34
Infested (%)	49/100	44/100
Grubs/head (range)	0–12/2–43	0–22/6–42
Average grubs/head[a]	1.9–15.0	3.6/21.8
Reduction (%)	87	84

[a] Combined grub counts significant at 1% level.

Table 8.—Effectiveness of two concentrations of Bayer 21/199 suspensions on calves, yearlings, and cows.[a]

Criteria	Counts by Palpation 1958			
	February 7		March 7	
	0.5%/ Control	0.25%/ Control	0.5%/ Control	0.25%/ Control
Calves				
No. sampled	11/10	5/4	10/10	4/3
Infested (%)	36/40	80/75	60/70	75/67
Grubs/head (range)	0–8/0–6	0–31/0–29	0–27/0–31	0–36/0–22
Average grubs/head	1.9–1.3	12.4/14.2	8.1/12.4	18.8/9.3
Reduction (%)	0	13	35	0
Yearlings[b]				
No. sampled	37/49	—	36/48	—
Infested (%)	43/45	—	28/79	—
Grubs/head (range)	0–16/0–5	—	0–18/0–13	—
Average grubs/head	1.3/1.2	—	3.5/4.6	—
Reduction (%)	0		24	
Cows[b]				
No. sampled	36/35	43/35	37/31	46/31
Infested (%)	14/31	19/31	24/55	37/55
Grubs/head (range)	0–6/0–8	0–4/0–8	0–9/0–18	0–9/0–18
Average grubs/head	0.3–0.7	0.4/0.7	0.7/2.0	1.2/2.0
Reduction (%)	57	43	65	40

[a] Commercial herds in central South Dakota sprayed November 21–22, 1957.
[b] Irregular pressures and poor holding facilities.

Table 10.—Effectiveness of Bayer 21/199 and Dowco 109 (0.5% suspensions) sprayed on cattle[a] with varying degrees of coverage, November, 1958.

Criteria	Counts by Palpation	
	February (Treated/Control)	March (Treated/Control)
Bayer 21/199 (Calves, total coverage)		
No. sampled	44/39	45/39
Infested (%)	27/97	33/95
Grubs/head (range)	0–16/0–34	0–11/0–26
Average grubs/head	1.0–15.8	1.0/10.6
Reduction (%)	94	91
Bayer 21/199 (Cows, backs only)		
No. sampled	53/47	55/46
Infested (%)	21/23	33/17
Grubs/head (range)	0–4/0–8	0–14/0–11
Average grubs/head	0.32/0.42	1.2/0.48
Reduction (%)	24	—
Dowco 109 (Yearlings, backs only)		
No. sampled	15/10	15/10
Infested (%)	33/50	53/70
Grubs/head (range)	0–13/0–17	0–7/0–18
Average grubs/head	1.24/4.40	1.4/5.9
Reduction (%)	72	76

[a] Experiment Station herds, central and western South Dakota.

treated, but the yearlings and cows were sprayed under poor holding conditions and with irregular pressures. The low grub populations among the yearlings and cows, as well as the unfavorable conditions of treatment, may provide part of the explanation for the indifferent control achieved. No explanation is offered for the failure among the calves.

The grub season of 1958–59 was the fifth year of work reported here. Yard tests at Brookings (table 9) were conducted with Bayer 21/199 and with Dowco 109. An attempt was made to compare suspension and emulsion formulations of Bayer 21/199, as well as applications to the backs only versus total coverage. An attempt was also made to compare this material with a corresponding formulation and concentration of Dowco 109. No practical differences in degree of control (94% to 97%) among the different formulations or degrees of coverage

Table 9.—Infestation, erythrocyte cholinesterase, and weight gain following various 0.5% emulsion or suspension spray treatments of steer calves, administered to backs only or to entire body surface November 22, 1958. Brookings, S. Dak.

Criteria	Treatment[a]				
	Bayer 21/199			Dowco 109	Untreated
	Suspension		Emulsion (Total Coverage)	Suspension (Total Coverage)	
	To Backs Only	Total Coverage			
Infestation					
Calves infested	1	3	4	4	4
Grubs/calf (range)	0–4	0–1	0–2	0–46	0–38
Average grubs/calf	1	0.8	1.2	16.2	22.2
Reduction (%)	96	97	94	27	—
Erythrocyte cholinesterase					
December 9, 1958 (17th day)					
Range[b]	0.24–0.31	0.18–0.24	0.16–0.30	0.32–0.47	0.28–0.50
Average[b]	0.26	0.20	0.23	0.38	0.36
Activity (% initial)	59	50	53	86	92
January 23, 1959 (62nd day)					
Range[b]	0.48–0.64	0.32–0.52	0.30–0.76	0.40–0.59	0.39–0.58
Average[b]	0.56	0.43	0.45	0.47	0.51
Activity (% initial)	129	106	104	107	131
Average weight gain/calf					
Initial weight (lb.)	440	386	434	430	420
Daily gain (lb.)[c]	1.51	1.43	1.43	1.45	1.44
Per cent of initial weight	44	47	41	43	44

[a] Four calves used in each category.
[b] ΔpH units/hour.
[c] Not significant at 5% level. Test ran for 128 days.

of Bayer 21/199 were noted. The low degree of control achieved with Dowco 109 at 0.5% was surprising when compared with the 100% control shown in table 6, where this material was used at the 0.75% dosage. On the 17th day after treatment moderate depression of erythrocyte cholinesterase activity was found in the Bayer 21/199 treatments with only very slight evidence of depression with Dowco 109. Though the total coverage treatments of Bayer 21/199 provided greater depression than the back-only treatment, the difference in activity was not as great as was expected. No outstanding differences in weight gains were noted.

Field tests in central and western South Dakota in 1958–59 (table 10) with Bayer 21/199 and with Dowco 109 involved larger numbers of animals than in the Brookings yard tests. The 91% to 94% control achieved by the complete coverage with Bayer 21/199 on calves may be considered very good. The results of the application of this material to the backs only of cows has little meaning if the low infestation in the untreated checks is considered. The results of the application of Dowco 109 to the backs only of calves appears surprisingly good when compared with the complete coverage as shown in table 9. The infestation, however, was rather low. Both species of cattle grubs were present among specimens extracted throughout these studies.

DISCUSSION.—The data presented in this five-year study provide the basis for some clear decisions, but they also introduce elements of uncertainty. The *in vivo* activity of the several chemicals derived from *in vitro* studies on oesophageal-stage larve was not sufficiently great to warrant further work on these compounds when the availability of the several commercial organophosphate materials is considered. This does not mean that at some future time it would be inappropriate to investigate analogs further or other formulations of some of these materials. The failure of di-n-butyl tin dilaurate is in agreement with the results of Cobbett *et al* (1957).

The results with ronnel reported herein (tables 4 and 5) support the efficacy of this material except (table 5) where infestation levels are less than one grub per head. The studies with dimethoate, oral as well as intramuscular, indicate high efficacy for this material, but the accompanying severe depression of cholinesterase activity (table 6), coupled with at least initial weight gain deceleration, was unfavorable.

The studies with Dowco 109 are less conclusive than those with ronnel. The results shown in table 6 indicate a very high degree of control coupled with no cholinesterase depression. The data in tables 9 and 10 suggest that considerable reduction in effectiveness of this material may be expected with reduced dosage or coverage.

The studies with Bayer 21/199 show considerable variation. At one extreme are the excellent results shown in tables 9 and 10 (calves); at the other extreme are the poor results shown in tables 8 and 10 (calves, yearlings and cows); while in table 7 (calves) moderate results for this compound are indicated. While poor spraying efficiency may explain the poor results shown for the yearlings and cows in table 8, the calves of this group were throroughly covered. These data suggest that while Bayer 21/199 is of great practical value, the development of a material that would provide a high level of control more consistently would be highly advantageous.

The weight-gain data of tables 3, 4, 6, and 9 do not provide evidence of a relationship between grub control and weight-gaining ability of calves maintained under dry-lot conditions.

It should be noted that, in general, the degree of grub control appears to be better in experiments involving heavily infested animals than in lightly infested animals. In this connection the superior performance of the insecticides applied to calves (tables 5, 8, 10) may be explained in part by the normally higher grub numbers in the calves, as well as by other differences that may be attributable to the age of the animals.

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Insecticidal Baits for Control of the Imported Fire Ant, *Solenopsis saevissima richteri*[1]

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ABSTRACT

Baits containing tankage, fish meal, ground insect bodies, processed vegetable (cooking) oils, digested corn and soybean protein plus fat (Staley SPIB FA-19C & 39), peanut butter, and certain other mixtures high in protein and/or fat were the most attractive of more than 400 formulations offered the imported fire ant (*Solenopsis saevissima* v. *richteri* Forel), in laboratory and field experiments. The insecticides heptachlor and Kepone® (1,2,3,5,6,7,8,9,10,10-decachlorotetracyclo [5.2.1.$0^{2,6}$.$0^{3,9}$.$0^{5,8}$] decan 4-one) were acceptable to the ant and effective in control to a greater degree than any of the other 27 materials studied. Peanut butter containing 0.125% Kepone was the most effective bait used in small- and large-plot field experiments. When this bait was applied at the rate of 4 or 6 pounds per acre, 100% of the fire ant colonies were eliminated. The peanut butter bait was injected into soda straws with a grease gun to facilitate application in the field. Other promising Kepone baits were prepared as pellets from equal parts of peanut butter and peanut meal and as polymerized granules of peanut butter.

The imported fire ant, *Solenopsis saevissima* v. *richteri* Forel, is a major nuisance, and it causes economic damage in some situations. The mounds interfere with the operation of farm machinery, and in heavily infested areas the ants inflict stings on field workers. Control of the imported fire ant is necessary on certain croplands, pastures, and lawns. Effective control methods have been developed by Blake *et al.* (1959). When these control measures are used in open fields, no damage to wildlife or domestic animals has been observed. Broadcast treatment of insecticides on large tracts of wildlife land has resulted in heavy mortality of these animals (Arant *et al.* 1958, Clawson & Baker 1959).

In 1957 experiments were begun to develop low-cost control with baits containing toxicants in amounts that would not be harmful to wildlife, beneficial insects, or other inhabitants of the treated area. The work was continued during 1958 and 1959.

PROCEDURE.—The experiments reported herein were conducted at the Main Experiment Station, Auburn; Black Belt Substation, Marion Junction; and Lower Coastal Plain Substation, Camden, of the Auburn University Agricultural Experiment Station.

Observations on fire ants in the field indicate that every foot of land in a generally infested area is foraged industriously. Therefore, experiments were conducted on an area-broadcast basis in all tests except those for purely exploratory purposes. In the exploratory tests, captive laboratory colonies or large, active mounds in the field were used.

Laboratory Studies.—Since this ant feeds principally upon insects (Hays & Hays 1959), a logical hypothesis indicated the use of a bait high in fat and protein with a slow-acting toxicant that would not repel the ants. Since insects normally eaten were not usually available in sufficient quantities for use in baits, attention was given to other materials that would be readily carried to the mound and consumed. A total of 68 solid foods and 36 liquids was screened for acceptability and attractiveness to the ants. Small ant colonies were established in the laboratory to test the effectiveness of prospective bait materials. Each colony was confined in a glass battery jar that was surrounded by water to prevent escape of the ants. Colonies were held 2 to 3 days without food before testing was begun, and an interval of 4 days was allowed between subsequent tests. To determine acceptability of a material, a small petri dish containing a known quantity of the material was placed on a mound. The receptacle was re-weighed 6 hours later to determine the amount of material taken by the ants. Observations were made at hourly intervals for 6 hours on the numbers of ants attracted to the material. Each material was tested separately on four colonies.

Mound Treatments in the Field.—Experiments were conducted in the field on strong ant colonies to determine the acceptability of combinations of promising materials under natural conditions. A 10-gram lot of bait was placed near a mound on a 6-inch square of aluminum foil. One material was used per mound with treatments replicated four times. Three observations were made at 4-hour intervals. The number of ants visiting the bait was recorded during the first and second observations, and the total amount of bait taken was recorded at the third observation. A total of 108 different baits was tested.

Using the most attractive baits, 29 toxicants were tested for acceptability and toxicity when incorporated into these baits. Each toxicant was used at rates of 1.0%, 0.5%, 0.25%, 0.125%, 0.0625%, and 0.03125%, respectively. Each treatment was replicated four times on active mounds that had not been used previously. A 10-gram lot of each bait was placed near a mound on a 6-inch square of aluminum foil. Observations were made every 4 hours, and the number of ants visiting the bait was recorded. The amount of bait taken was recorded at the third observation. A final check was made 2 weeks after treatment by breaking into the mound to determine if the colony was active. If no ants were observed the soil was excavated to a depth of approximately 1 foot below the mound in search of living ants. If no live ants were found, the bait was considered effective.

Area Treatments.—Small-plot field experiments were conducted on heavily infested areas to determine the attractiveness and efficiency of various bait-toxicant mixtures, the amount of bait required, and the best method of application for control of imported fire ants. Each treatment was replicated four times on 1/10-acre

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Table 1.—Acceptability and attractiveness of solid and liquid food products to the imported fire ant in the laboratory, 1957.

Bait Material	Acceptance Rating[a]
Dry	
Alfalfa meal, dry, commercial feed grade	0
Alfalfa meal, 4 parts; cooking oil, 1 part	1
Banana, mashed	1
Banana, peel, ground	0
Beef fat, ground, fresh	2
Beer, stale on blotter paper	1
Blood, beef, whole on blotter paper	0
Bacon, fresh	3
Beef, dry, ground	0
Beef, bouillon on blotter paper	1
Corn meal, dry	0
Corn meal, 4 parts; cooking oil, 1 part	2
Corn meal, 4 parts; honey, 1 part	1
Corn meal, 4 parts; cod liver oil, 1 part	2
Corn meal, 4 parts; whole beef blood, 1 part	0
Casein, dry, commercial grade	0
Cracklings, ground	3
Corn, coarse crushed	0
Cotton seed meal, commercial feed grade	0
Chicken feed, dry laying mash	2
Dog food, dry meal	1
Dog food, dry pellets	1
Dog food, canned, ground	1
Dried apple, ground	1
Egg, whole on blotter paper	2
Fish meal, dry commercial feed grade	1
Fish meal, 4 parts; cooking oil, 1 part	2
Honey, on blotter paper	0
Honey, equal parts with water on blotter paper	1
Honey, 1 part; wheat bran, 4 parts	1
Hamburger meat, fresh	2
Insect pulp, ground fresh insects	3
Insect extract, extracted in cooking oil	3
Insect extract, extracted with acetone and evaporated	2
Insect meal, insects cooked and ground	1
Insect meal, 4 parts; cooking oil, 1 part	3
Oats, ground, dry	0
Oats, ground, 4 parts; cooking oil, 1 part	1
Pineapple, ground, canned	0
Peanuts, raw, ground	3
Peanuts, raw, whole	3
Peanuts, raw, ground with hulls	3
Peanut meal, dry, commercial feed grade	1
Peanut butter, commercial grade	3
Peanut meal, 4 parts; cooking oil, 1 part	2
Peanuts, parched, coarsely ground	3
Pork fat, ground, fresh	3
Raisins, ground	0
Sausage, fresh pork	3
Sawdust, 5 parts; cooking oil, 1 part	2
Semen, swine, fresh, whole	1
Sugar, powdered, dry	0
Sugar, granular, dry	0
Sugar, equal parts with water on blotter paper	1
Soybean meal, dry, commercial feed grade	1
Soybean meal, 4 parts; cooking oil, 1 part	3
Tankage (50% protein, meat and bone scraps), dry	2
Tankage, 4 parts; cooking oil, 1 part	3
Wheat bran, dry, commercial feed grade	1
Wheat bran, 4 parts; cooking oil, 1 part	3
Whey, dry, commercial feed grade	0
A. E. Staley Co., SPIB FA-31, high protein corn residue	3
A. E. Staley Co., SPIB FA-33, high protein corn residue	1
A. E. Staley Co., SPIB FA-35, condensation of amines containing residue	0
A. E. Staley Co., SPIB FA-38, high protein B_{12} containing residue	0
A. E. Staley Co., SPIB FA-39, soybean protein plus fat	3
A. E. Staley Co., SPIB FA-40, soybean phosphatide containing soya protein	0
A. E. Staley Co., SPIB FA-45, corn protein amines acids	0
Liquid	
Butter, melted, table grade	2
Bacon drippings	3
Corn oil, processed	3
Corn oil, crude	3
Coca Cola syrup, commercial drink grade	1
Cod liver oil, commercial drug grade	1
Cotton seed oil, processed	3
Cherry syrup, commercial drink grade	1
Grape syrup, commercial drink grade	1
Lime syrup, commercial drink grade	1
Lard, pure, melted	3
Orange syrup, commercial drink grade	1
Peppermint oil	0
Peanut oil, processed	3
Peanut oil, crude	3
Root beer, commercial drink grade	1
Syrup, commercial cane table syrup	1
Vegetable oil, commercial cooking oil	3
A. E. Staley Co., SPIB FA-2, corn protein hydrolysate	0
A. E. Staley Co., SPIB FA-7, corn protein hydrolysate	0
A. E. Staley Co., SPIB FA-19, enzyme digested corn protein, digestion variable	3
A. E. Staley Co., SPIB FA-19A, enzyme digested corn protein, digestion variable	3
A. E. Staley Co., SPIB FA-19C, enzyme digested corn protein, digestion variable	3
A. E. Staley Co., SPIB FA-20, enzyme digested corn protein, digestion variable	0
A. E. Staley Co., SPIB FA-20A, enzyme digested corn protein, digestion variable	1
A. E. Staley Co., SPIB FA-20C, enzyme digested corn protein, digestion variable	0
A. E. Staley Co., SPIB FA-30, soybean phosphatides	0
A. E. Staley Co., SPIB FA-32, high protein corn residue	0
A. E. Staley Co., SPIB FA-34, high protein corn residue	0
A. E. Staley Co., SPIB FA-37, a fatty acid containing product	0
A. E. Staley Co., SPIF FA-41, corn oil sterols	0
A. E. Staley Co., SPIB FA-42, soya oil fatty acids	0
A. E. Staley Co., SPIB FA-43, corn oil distillates	0
A. E. Staley Co., SPIB FA-44, soya oil distillates	0
A. E. Staley Co., SPIB FA-46, corn protein hydrolyzate	0
A. E. Staley Co., SPIB FA-47, fatty acid amines	0

[a] 3, maximum acceptance; 0, no acceptance.

plots and plots were randomized. Tests were conducted with 312 different bait-toxicant mixtures. In preparing the baits, different procedures were used for incorporating the toxicant. In one series of tests, the toxicant was dissolved in acetone before mixing with the bait; in another series, it was mixed directly with the liquid or solid bait; in still other tests, the toxicant was dissolved or emulsified in a liquid portion of the bait, which was then mixed with a dry component. All baits were applied within 96 hours after mixing.

Large-scale field experiments were conducted to test further the effectiveness of the more promising baits. One hundred and twelve different treatments were replicated four times on plots ranging from ½ acre to 10 acres in size. The baits were prepared in the laboratory or manufactured commercially. All baits were either broadcast or distributed evenly over the treated area. To facilitate dis-

Table 2.—Acceptability by the imported fire ant of tankage-cooking oil baits containing certain insecticides at the listed concentration, 1957.

Insecticide	Per Cent Maximum Acceptable Concentration	Bait Acceptance Rating[a]	Per Cent Mounds Inactivated
Aldrin	0.25	2	75
Anticide[b]	0.5	2	0
Chlordane	0.25	1	50
Diazinon®[c]	0.25	1	50
Dieldrin	0.125	1	25
Dipterex®[d]	0.125	1	0
DDT	0.062	0	0
Endrin	0.062	1	0
Guthion®[e]	0.25	1	25
Heptachlor	0.042	2	90
Delnav®[f]	0.062	1	0
Kepone	0.125	3	100
Kepone w.p.	0.062	0	0
Korlan®[g]	0.25	1	25
Lindane	0.5	1	25
Methoxychlor	0.25	1	0
Malathion	0.062	0	0
Phosdrin®[h]	0.062	0	0
Parathion	0.125	1	50
Sodium arsenate	0.5	3	0
Sodium arsenite	0.5	2	0
Sodium fluoride	0.25	1	0
Sodium fluoroacetate (1080)	0.5	3	0
Sevin®[i]	0.25	1	50
Tartar emetic	0.5	2	9
Toxaphene	0.25	1	25
Thuricide®[j]	0.5	3	0
Thallium sulfate	0.25	1	0

[a] 3, maximum acceptance; 0, no acceptance. Four replications.
[b] Stabilized rotenoids, octo (CDE) insecticide activator, propellent gases, wetting agent, and petroleum oil.
[c] *O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidyl) phosphorothioate.
[d] *O,O*-dimethyl 1-hydroxy-2,2,2-trichlorethylphosphonate.
[e] *O,O*-dimethyl *S*-(1,2,3-benzotriazinyl-4-keto)-3-methyl phosphorodithioate.
[f] 2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethylphosphorodithioate).
[g] *O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate.
[h] *O,O*-dimethyl *O*-1-methoxycarbonyl-1-propen-2-yl phosphate.
[i] (1-naphthyl-*N*-methyl carbamate).
[j] *Bacillus thuringiensis*.

tribution, peanut butter formulations were injected into soda straws with a grease gun, and the filled straws were distributed in the field by hand or with a manually operated applicator. Counts of active ant mounds were made on each plot prior to treatment and again 30 days after treatment. Intermittent observations on ant activity were made between the time of treatment and the final infestation counts.

Observations were made in all field experiments to determine the effects of poison baits on birds, small rodents, other wildlife, and cattle.

Results.—The imported fire ant was attracted to and accepted only those bait materials that contained considerable quantities of protein and oil. Most oils were accepted alone or in combination with solids. Some of the mixtures were more readily accepted than either the oils or solids alone. In mixing oil with a solid bait, it was important to use only enough oil to ensure uniform distribution throughout the mixture. Too much oil resulted in a sticky or lumpy bait, which was difficult to distribute.

Laboratory Studies.—Detailed information on acceptability of 104 bait materials by laboratory colonies of ants is presented in table 1. Tankage, peanut meal, and peanut butter were accepted more readily than the other solid materials. All the cooking oils (corn, cotton seed, peanut, and soybean) were accepted readily, and no preference was shown for any one. SPIB FA-31, SPIB FA-39, SPIB FA-19C, and several other Staley products were also accepted. The most attractive material of all those tested was peanut butter. The ants accepted peanut butter in all cases and transported large amounts of it to the mounds. Some of this material was found in the mound stored in cells.

Table 3.—Effectiveness of area treatments with the more promising insecticidal baits for fire ant control, 1959.

Bait Formulation	Insecticide and Per Cent Concentration	Bait per Acre (Lb.)	Per Cent of Mounds Inactivated[a]
Tankage, fish meal, and cooking oil	Heptachlor 0.042	50	78.9
Tankage, fish meal, and cooking oil	Kepone 0.062	50	91.7
A. E. Staley Co. bait SPIB FA-19C & 39	Heptachlor 0.042	50	72.2
Peanut butter in soda straws	Kepone 0.125	4	100.0
Peanut butter in soda straws	Kepone 0.125	6	100.0
Nontreated check	—	—	0.0

[a] Two replications, 10-acre plots.

Mound Treatments in the Field.—In experiments in the field with 108 combinations of baits, tankage and oil, wheat bran and oil, and peanut butter were the most readily accepted of all combinations tested. The Staley materials, SPIB FA-31, SPIB FA-39, and SPIB FA-19C, were also accepted and transported to the mound.

Information is presented in table 2 on fire ant acceptance of insecticides in tankage–oil baits applied to mounds in the field. Several of the toxicants tested (sodium arsenate and sodium fluoroacetate) were very acceptable to the ant and were taken readily, but no kill was obtained. Heptachlor and Kepone® (1,2,3,5,6,7,8,9,10,10-decachlorotetracyclo [5.2.1.$0^{2,6}$.$0^{3,9}$.$0^{5,8}$] decan-4-one) were the most effective materials; however, heptachlor acted as a repellent at concentrations greater than 0.042%. The worker ants were observed to stagger and exhibit other uncoordinated movements while taking heptachlor bait, and were found dead in the area. Apparently the entire colony sensed danger, especially when the higher concentrations of heptachlor were used. Much movement and relocation of colonies were noted following area treatment with heptachlor. This movement made it difficult to take accurate infestation counts. The optimum heptachlor concentration appeared to be 0.042%; in lower concentrations the percentage kill was reduced. Kepone at 0.125% resulted in the most effective control of any insecticide tested. This slow-acting stomach poison allowed the ants to take the bait in considerable quantities and transport it to the mound. The maximum kill resulting from Kepone usually came from 7 to 15 days after bait application.

The most satisfactory baits were formed when the insecticide was incorporated into oil, and this material in turn was mixed with the tankage. Acceptable baits were taken whenever the ants were active, but they were taken most readily on warm days. When baits were placed on the mounds and the colony disturbed, the ants often carried an undesirable material into the mound. Such materials were discarded later or sealed in cells in the mound.

Area Treatments.—Of the 312 bait combinations tested

Table 4.—Effectiveness of different concentrations of Kepone in peanut-butter bait applied at the rate of 6 pounds per acre for control of imported fire ant. Camden, Alabama, 1959.

Per Cent Kepone	Number of Active Mounds per Acre[a]	
	Prior to Treatment	30 Days After Treatment
0.1250	33.8	0.0
0.0937	37.3	13.5
0.0625	42.0	13.9
0.0417	32.7	40.0
0.0 (Check)	40.0	40.5

[a] Four replications.

in small-plot field experiments for attractiveness and effectiveness, the ants showed preference for tankage, peanut meal, and peanut butter baits containing heptachlor or Kepone as the toxicant. The most effective method of incorporating the toxicant with the bait consisted of dissolving the toxicant in acetone and blending the solution with the dry material or with the peanut butter.

All the 112 bait combinations used in large-scale field experiments annihilated some colonies. However, only three baits proved sufficiently effective to provide satisfactory control (table 3). One of these baits consisted of three parts tankage, one part fish meal, and one part cooking oil, plus 0.042% heptachlor or 0.062% Kepone. Kepone was more effective than heptachlor, but neither eliminated 100% of the colonies in the treated area. The optimum rate of broadcast treatment was 50 pounds of prepared bait per acre. Another moderately effective bait consisted of soybean protein and enzyme digested corn protein plus fat (SPIB FA-39 and 19C) prepared by the A. E. Staley Company. The bait also contained 0.042% heptachlor. It was about equal to the tankage–fish meal bait in effectiveness. Single or two applications at 3-week intervals, each at the rate of 50 pounds per acre, destroyed 72.2% of the colonies. The third and most effective material was peanut butter containing 0.125% Kepone, applied at the rate of 4 or 6 pounds per acre. This bait eliminated 100% of the ant colonies (table 3). Replicated experiments in 1959 proved that lower concentrations were ineffective (table 4).

Peanut butter bait containing 0.125% Kepone was 100% effective against imported fire ants when applied at rates of 4 to 10 pounds per acre (table 5). The 6-pound rate appeared to be optimum, since some difficulty was encountered in getting even distribution at the 4-pound rate.

There was no difference in the efficiency of baits distributed in soda straws varying in length from 1.6 to 8 inches. However, the shorter lengths were easier to distribute in a uniform pattern than the long straws. The shorter lengths were also less attractive to animals.

Although peanut butter baits were more readily accepted by the ants than any other materials tested, application of these baits in the field was difficult. It was found that the bait could be handled and distributed satisfactorily in soda straws. Formulations used successfully in the straws included various concentrations of Kepone in peanut butter and in mixtures containing 97% peanut butter and 3% paraffin or 3% agar agar. Pellets

Table 5.—Effectiveness of 0.125% Kepone–peanut butter bait at different rates per acre for control of imported fire ant. Camden, Alabama, 1959.

Bait per Acre (Lb.)	Active Mounds per Acre			
	½-Acre Plots		10-Acre Plots	
	No. Before Treatment[a]	No. 30 Days After Treatment[a]	No. Before Treatment[b]	No. 30 Days After Treatment
0 (check)	33.8	33.0	39.0	40.5
2	49.0	18.9	—	—
3	56.0	0.0	43.0	0.0
6	40.1	0.0	40.9	0.0
8	32.5	0.0	—	—
10	34.3	0.0	—	—

[a] Average of four replicates.
[b] Average of duplicate plots.

prepared from equal parts of peanut butter and peanut meal and polymerized granules of peanut butter were also used successfully. The paraffin, agar agar, and peanut meal increased the stability of the peanut butter at warm temperatures.

Ants were attracted in great numbers to peanut-butter bait and took considerable quantities of it in 12 hours or less. Immature forms in the colony were apparently fed large amounts of the bait almost immediately and other forms in the mound were fed later. When the ants began dying from Kepone baits, the workers opened the top of the mound and ejected the dead. The rate and amount of mortality could be measured by the numbers of dead ants observed on top and around the base of the mound. It was noted that the immature forms, small workers, major workers, and winged forms died in the order named. Usually several days elapsed between the initial kill and death of the winged forms.

In one experiment 70 cattle ate 160 pounds of Kepone-peanut butter bait distributed in 8-inch straws over 40 acres of pasture. Sixty pounds was consumed following an initial application, and 100 pounds was eaten following a repeat application 14 days later. There was no evidence of any ill effect on the animals. These cattle ate little, if any, of the bait when it was distributed in straws $2\frac{2}{3}$ inches in length. Careful observations in all experiments with Kepone revealed no evidence of loss to wildlife or domestic animals.

Additional research is needed on the effect of Kepone baits on wildlife and domestic animals. Emphasis should be on possible storage of Kepone in animal tissues and secretion of the material in the milk of dairy cows.

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Epoxidation of Aldrin and Heptachlor in Soils As Influenced by Autoclaving, Moisture, and Soil Types[1,2]

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Abstract

Aldrin was applied to Quartz sand, Plainfield sand, Carrington loam and muck soils in order to study its persistence and epoxidation. In addition autoclaved-wet and nonautoclaved dry sand and loam soils were used. Aldrin was readily transformed into dieldrin in a wet-nonautoclaved Carrington loam, but less rapidly so in a muck soil. The amounts of aldrin and dieldrin recovered from aldrin-treated Carrington loam soils were equal 3.3 months after treatment under laboratory conditions at 37° C. and 16 months after treatment under field conditions.

In soils containing a low number of microorganisms (autoclaved loam, Plainfield sand) or in dry soils the amount of dieldrin formed was small. After heptachlor had been applied to a Carrington loam it was found that this insecticide persisted slightly longer than aldrin, but the amount of heptachlorepoxide formed was smaller than that of dieldrin. A similar study on lindane persistence in a Miami silt loam is also reported.

Residue analyses for insecticides were originally done to detect the presence of the specific toxicant initially applied. However, such an analysis might now show the total amount of toxicants present. Certain insecticide breakdown products still have some toxic effect, yet are not detected by a specific analytical method for the applied toxicant. Two such insecticides are aldrin and heptachlor, since they are oxidized to their respective epoxides: dieldrin and heptachlorepoxide.

The conversion of aldrin to dieldrin in animal tissues was shown by Bann *et al.* (1956), and has also been demonstrated to occur in soils (Edwards *et al.* 1957, Gannon & Bigger 1958, Bollen *et al.* 1958, Lichtenstein & Schulz 1959a). Moreover dieldrin was found within crops grown in aldrin treated soils (Lichtenstein 1959). Heptachlor has also been shown to be converted to its epoxide in soils (Gannon & Bigger 1958).

Some factors which might affect the persistence and epoxidation of aldrin and heptachlor in soils were investigated under laboratory and field conditions.

Procedures.—1) *Laboratory Experiments.*—The effects of microorganisms and of moisture on the persistence and epoxidation of aldrin and heptachlor were ascertained in the following experiments:

a) 140 grams of freshly sampled Carrington loam (organic matter = 4.3%) were placed into each of 42 glass jars (capacity 500 ml., opening, 2″). After all jars were plugged with cotton one-third of them (14) were autoclaved at 15 lb. pressure and 121° C. for 7 hours daily for a period of 4 days. On the fifth day both the autoclaved soils and the nonautoclaved soils were treated with aldrin at a rate of 10 p.p.m. by pipetting measured amounts of the insecticide in an acetone solution onto the soil. The jars were then closed again by means of cotton plugs and the soil was mixed by rolling horizontally for 15 minutes on a specially designed apparatus (Lichtenstein & Schulz 1959a). Soil samples from six jars were then used for initial analyses, and the remainder were placed in an incubator at 37° C. During the experiment (112 days) the autoclaved soils and one-half of the nonautoclaved soils were watered once a week with sterilized distilled water to hold the moisture content close to field conditions. The other half of the nonautoclaved soils was not watered ("dry nonautoclaved"). Two samples of each series (wet nonautoclaved, wet autoclaved and "dry" nonautoclaved) were taken 7, 14, 28, 56, 84 and 112 days after treatment for analyses.

b) The identical experiment as described above was repeated with heptachlor.

In addition aldrin was added to water extracts of a Carrington loam (Thornton's Standardized Media) to see if a conversion of aldrin to dieldrin took place under such conditions.

c) In a third experiment a Plainfield sand (organic matter = 0.8%) was treated with aldrin at a rate of 10 p.p.m. Otherwise the same procedure as described above was employed.

d) To study the effect of a Quartz sand as well as three soil types, the same volumes (148 ml.) of a Quartz sand (238 gm.), Plainfield sand (176 gm.), Carrington loam (140 gm.) and a muck soil (110 gm.) (organic matter = 40%) were placed into 500 ml. jars and treated with aldrin at a rate of 10 p.p.m. (dry weight basis). The jars were plugged with cotton and the soil was mixed as described previously. Two soil samples of each type were used for initial analyses and the remainder were placed in an incubator at 37° C. All samples were kept moist by watering once a week with sterile distilled water. After 56 days the replicated Quartz sand samples as well as the replicated three soil types were analyzed for aldrin and dieldrin.

e) In another experiment lindane was applied to a Miami silt loam in 500-ml. glass jars as described previously, and the soils were held in an incubator at 46° C. Two replicates each of wet nonautoclaved, wet autoclaved and "dry" nonautoclaved soil were analyzed 14, 28 and 56 days after soil treatment.

Determinations of organic matter content of the soils as well as pH measurements were made periodically. The numbers of microorganisms (bacteria, fungi and streptomyces) in the soils were determined at the beginning of the experiments and at various intervals thereafter.

2) *Field experiments.*—In May, 1958, six plots (Carrington loam) of 30′×40′ were staked out, leaving 20′

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buffer strips in between. Two plots were treated with aldrin at a rate of 25 lb./5″ acre and two with heptachlor at a rate of 25 lb./5″ acre. The other two plots were used as controls. The insecticidal application involved a thorough mixing of a measured amount of an emulsifiable concentrate with 20 gallons of water. Each experimental plot was subdivided into five 6′×40′ strips in the south-north direction and into five 8′×30′ strips in the east-west direction. Two-gallon quantities of the diluted emulsion were then spread as uniformly as possible with a sprinkling can over each of the 10 strips. This crosswise treatment was used to attain a more uniform application.

The Shell Chemical Corporation and the Velsicol Chemical Corporation provided solvent-emulsifer solutions, which differed from the aldrin or heptachlor formulations used only in that the insecticide was absent. These solutions were used for "treatment" of the two control plots. All plots were rototilled to a depth of 4″ to 5″ immediately after treatment. Soil samples were collected initially and then 2, 4, 12 and 17 months thereafter. Sampling was done with a soil auger and 40 cores ($\frac{3}{4}$ inch diameter, 6 inches long) were collected from each plot. The area of the experimental plot was reduced to 24′×34′ for sampling, leaving a 3′ strip between the boundaries of the treated plot and the sampling area.

During the two summer periods (1958 and 1959), various vegetable crops were grown on these plots.

3) *Analytical Methods.*—The handling of the soil samples as well as their extraction was done as described by Lichtenstein & Schulz (1959a and b). The extraction solvents used were a mixture of redistilled hexane and isopropanol (2:1) for aldrin-treated soils and a mixture of colorimetric pentane and acetone (4:1) for heptachlor-treated soils.

The isopropanol or the acetone were removed from the soil extracts by washing with water. The alcohol or acetone free phase was then dried over anhydrous sodium sulfate.

Since extracts of aldrin-treated soils were analyzed for aldrin and dieldrin, cleanup as well as separation of the two insecticides was done by using a column (8″×1″) of magnesiumoxide-celite (1:1). After the extract containing aldrin and dieldrin had been added to the column, aldrin was eluted with 100 ml. of redistilled hexane. Dieldrin could then be washed from the column with an additional 600 ml. of the same solvent. Aldrin and dieldrin fractions were analyzed according to the method of O'Donnell *et al.* (1954, 1955). Known amounts of aldrin added to soils were recovered to an extent of 93% to 97% and known amounts of dieldrin to an extent of 90% to 94%. A good separation of aldrin and dieldrin was obtained when both chemicals were used in recovery analyses.

Extracts from heptachlor-treated soils were analyzed for heptachlor and heptachlorepoxide. The cleanup of the extracts as well as the separation of the two insecticides was done as described by Lichtenstein & Polivka (1959). The analysis of the two insecticides were performed according to the Polen-Silverman method (Polen & Silverman 1952, Ordas *et al.* 1956). Known amounts of heptachlor or heptachlorepoxide added to soils were separately recovered to an extent of 90% to 96%.

Since the yellow color developed for heptachlorepoxide (absorption peak at 415 mμ.) is similar to colors developed by interfering substances, extreme care had to be taken to obtain reliable and low values for control samples. In many cases absorption curves for the heptachlorepoxide sample were established in order to check if the yellow color obtained had an absorption peak of 415 mμ.

Each analysis was run in duplicate using a soil blank for the determination of apparent insecticide. In addition known amounts of insecticides were added to insecticide-free samples. This action permitted a check of the analytical procedure for each analysis done. The unknowns, after the values for apparent insecticide had been subtracted, were calculated on the basis of the values obtained for the known amounts. Results were expressed in parts per million based on the dry weight of the soil under investigation.

As residue data for lindane obtained by bioassay seem to present a more realistic assessment of actual toxicants present (Lichtenstein & Schulz 1959a), data presented for lindane in this paper are those obtained by using a bioassay with *Drosophila melanogaster* Meig as the test insect. A direct feeding method as described by Edwards *et al.* 1957 was employed.

In addition to the chemical and biological methods for analyses, ascending paper chromatography was used as described by Mitchell (1957). R_f values for the different insecticides were established by using fractions of soil extracts containing either aldrin, dieldrin, heptachlor or heptachlorepoxide and compared with R_f values obtained from the known compounds.

RESULTS AND DISCUSSION.—1) LABORATORY EXPERIMENTS.—In those experiments in which autoclaved soils were used, the numbers of microorganisms in soils were determined and compared with those in nonautoclaved soils. Though the soils had been subjected to autoclaving for 4 days, initial counts still revealed the presence of small numbers of microorganisms (table 1). In addition the populations of microorganisms built up over a period of 56 days.

Table 1.—Numbers of microorganisms found in an autoclaved and a nonautoclaved Miami silt loam soil treated with lindane at 10 p.p.m.

Days After Autoclaving	Microorganisms per Gram of Dry Soil	
	Autoclaved[a]	Nonautoclaved
	Bacteria	
0	333	1,270,000
7	17,700	3,330,000
14	40,300	1,900,000
28	62,300	2,600,000
56	1,100,000	2,200,000
	Streptomyces	
0	200	1,100,000
7	22,000	2,700,000
14	33,700	3,100,000
28	82,600	2,800,000
56	1,600,000	1,600,000
	Fungi	
0	<100	2,300
7	<100	700
14	>300,000	<1,000
28	>300,000	<1,000
56	300,000	800

[a] All soils autoclaved for 4 days, 7 hours daily.

Table 2.—Residues of 1) aldrin (A) and dieldrin (D) and 2) heptachlor (H) and heptachlorepoxide (HO) found in a Carrington loam 112 days after treatment (10 p.p.m.) with aldrin or heptachlor.[a]

	WET[b]	WET-ST[c]	DRY[d]
1) *In per cent of initial aldrin recovery*			
A	21.7	53.5	82.6
D	24.0	8.0	5.3
Total	45.7	61.5	87.9
2) *In per cent of initial heptachlor recovery*			
H	33.4	62.5	39.2
HO	6.0	2.1	2.6
Total	39.4	64.6	41.8

[a] Temperature, 37° C.
[b] Nonautoclaved wet soil.
[c] Autoclaved wet soil.
[d] Nonautoclaved dry soil.

Table 3.—Recoveries of aldrin (A) and dieldrin (D) residues from two soil types 112 days after treatment with aldrin (10 p.p.m.).[a]

		WET[b]	WET-ST[c]	DRY[d]
P.P.M. in a Carrington loam				
Total: A+D		4.35±0.34	5.87±0.32	8.38±0.26
Per cent of total	A	47	87	95
	D	53	13	5
P.P.M. in a Plainfield sand				
Total: A+D		5.97±0.11	6.77±0.10	7.68±0.31
Per cent of total	A	93	97	96
	D	7	3	4

[a] Temperature, 37° F.
[b] Nonautoclaved wet soil.
[c] Autoclaved wet soil.
[d] Nonautoclaved dry soil.

Twenty-eight days after autoclaving a Carrington loam (treated with heptachlor at 10 p.p.m.) the numbers of bacteria per gram of dry soil were below 1000 as compared with 21,000,000 in a similar nonautoclaved sample. The respective figures for streptomyces were 3000 and 53,000,000. The numbers of fungi were approximately the same in all soil samples. When numbers of microorganisms were compared in two soil types at the start of the experiment, it was found that a Carrington loam contained 61 times more bacteria, 11 times more fungi and 48 times more streptomyces than the Plainfield sand.

All loam soils had a water content of 14% to 20%, based on the wet soil weight, throughout the duration of the experiments. However the "dry" nonautoclaved series dried out within 4 to 5 weeks after which no further water loss occurred in holding a 50-gm. soil sample at 55° C. for 24 hours.

The pH of the Carrington loam soils was similar in all soils used in one experiment and ranged from 5.9 to 7.2 in the aldrin-treated soils, and between 5.1 to 6.6 in the heptachlor-treated loam soils. Organic matter determinations were made of all soil samples to which aldrin had been added to a Carrington loam. It was found that there was no difference in organic matter content between the nonautoclaved, autoclaved or "dry" nonautoclaved soils.

The Plainfield sand was kept at a water level of 4% to 6% with the exception of the "dry" nonautoclaved series. When a water determination was made 2 weeks after the start of the experiment, the soil was dry by the criterion indicated above. The pH was similar in all aldrin-treated sand samples and ranged from 4.9 to 5.9.

Persistence and Epoxidation of Aldrin and Heptachlor in a Carrington Loam.—a) After aldrin had been applied (10 p.p.m.) to a Carrington loam it was found that the insecticide disappeared fastest in a wet nonautoclaved soil (tables 2 and 3). Only 2.06±0.05 p.p.m. of the initially-applied aldrin was present, 112 days after treatment. However, the amount of dieldrin in the soil increased with time and equaled that of aldrin 104 days after treatment (fig. 1a). One week later the amount of dieldrin was 2.29±0.27 p.p.m.

In an autoclaved soil, however, 5.11±0.17 p.p.m. of the applied aldrin was still present 112 days after treatment. The amount of dieldrin recovered was considerably smaller than in the nonautoclaved soil and amounted to only 0.76±0.14 p.p.m. In fact, the amount of dieldrin found during the first 56 days of the experiment was below 0.2 p.p.m. and increased slightly between the 56th and the 112th day. This coincides with a gradual buildup of the microorganisms in the soil.

Aldrin was most persistent in the soil that was not watered and consequently dried out. One hundred and twelve days after treatment 7.87±0.16 p.p.m. of the applied aldrin was recovered. Moreover the amount of dieldrin found was the smallest (0.51±0.10 p.p.m.) and was the same as that found 2 weeks after treatment when the soil had a water content of only 2.5%. When aldrin was added to water extracts of a Carrington loam (Thornton's Standard Media) no dieldrin formation was evident.

b) After heptachlor had been applied (10 p.p.m.) to a Carrington loam it was found that under all three experimental conditions (wet nonautoclaved soil, wet autoclaved soil and "dry" nonautoclaved soil) the amount of heptachlorepoxide formed was small (table 2). Paper chromatograms of the heptachlorepoxide fractions revealed the same R_f value as analytical grade heptachlorepoxide and the absorption curves of the heptachlorepoxide fractions had an absorption peak at 415 mμ.

In the wet nonautoclaved soil 3.62±0.07 p.p.m. of the originally applied heptachlor and 0.66±0.02 p.p.m. of its epoxide were recovered 112 days after soil treatment. A slight increase of heptachlorepoxide occurred as time progressed, though the rate of heptachlor epoxidation was lower than that for aldrin epoxidation (fig. 1a, 1b). As the rate of loss of heptachlor was smaller than that of aldrin the total amounts of toxicants (aldrin+dieldrin or heptachlor+heptachlorepoxide) recovered after 112 days were similar.

In the wet autoclaved loam, heptachlor was again more persistent than aldrin, but only 0.23±0.10 p.p.m. of heptachlorepoxide were found 112 days after soil treatment. However the loss of heptachlor was twice as great as the loss of aldrin in the loam soil, which was not watered and which dried out within the first 28 days. This loss might be related to the higher vapor pressure of heptachlor. A total of only 4.54±0.27 p.p.m. of heptachlor and heptachlorepoxide was recovered after 112 days as compared with a total of 8.38±0.26 p.p.m. of aldrin and dieldrin under identical conditions.

The amount of dieldrin recovered from the nonauto-

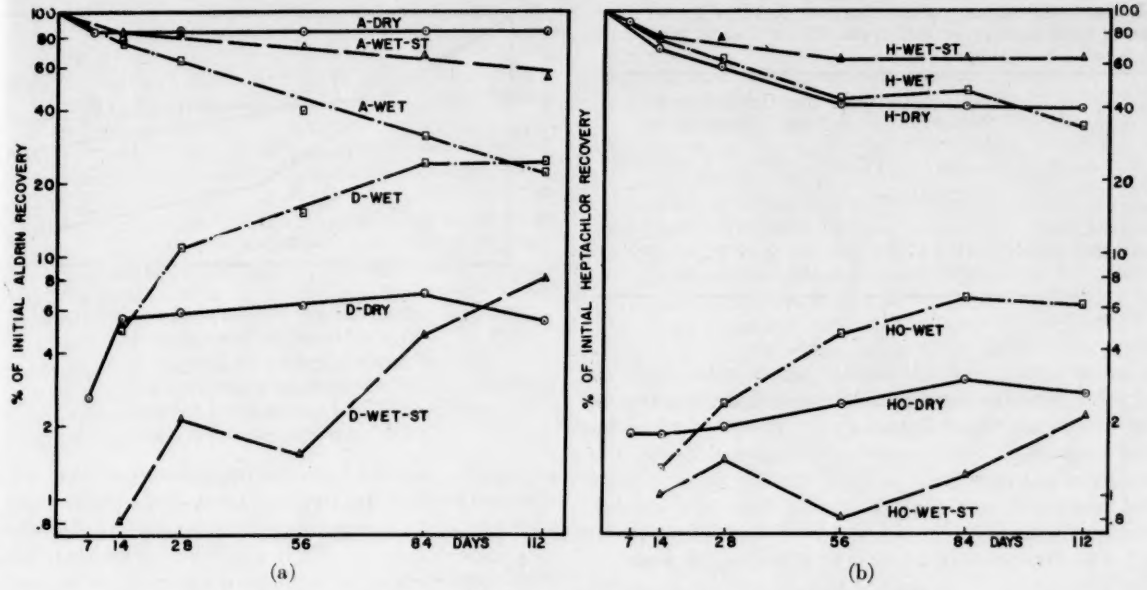

FIG. 1a.—Conversion of aldrin (A) to dieldrin (D) in a Carrington loam at 37° C.
Applied: aldrin 10 p.p.m.
WET = nonautoclaved wet soil
WET-ST = autoclaved wet soil
DRY = nonautoclaved dry soil

FIG. 1b.—Conversion of heptachlor (H) to heptachlorepoxide (HO) in a Carrington loam at 37° C.
Applied: heptachlor 10 p.p.m.
WET = nonautoclaved wet soil
WET-ST = autoclaved wet soil
DRY = nonautoclaved dry soil

claved soil 112 days after treatment was three times larger than that recovered from the autoclaved soil. The same ratio was found when recoveries of heptachlorepoxide from these two soils were compared, though the amounts of dieldrin recovered were in both cases four times larger than the amounts of heptachlorepoxide found (table 2).

c) *Persistence and Epoxidation of Aldrin in a Plainfield Sand.*—The differences in dieldrin formation from aldrin due to autoclaving or to nonwatering as noted previously in a loam soil were not evident with a Plainfield sand (table 3). In a wet sand aldrin was more persistent than in a wet loam and the amount of dieldrin formed was small (0.40 ± 0.07 p.p.m.) as compared with the loam soil (2.29 ± 0.27 p.p.m.). In fact, the amounts of dieldrin recovered from the sandy soil—112 days after treatment were similar under all three experimental conditions. Most of the aldrin was recovered from a dry sand, less from the autoclaved sand and the smallest amount from the wet nonautoclaved sand (fig. 2).

The total amount of aldrin and dieldrin recovered 112 days after soil treatment was slightly larger in the wet sandy soils than in the wet loamy soils and was similar in the dry Plainfield sand and dry Carrington loam.

The results seem to indicate that in soils having a low microorganism population or in dry soils where microorganisms are low in activity, aldrin was retained longer and none or only small amounts of dieldrin were formed from the aldrin present.

d) *Influence of Quartz Sand and Three Soil Types on the Persistence and Epoxidation of Aldrin.*—Fifty-six days after soil treatments with aldrin (table 4) practically all the toxicants had disappeared from the Quartz sand, possibly owing to the absence of any organic matter to which the insecticide could have been bound. However the three soil types used still contained a total (aldrin and dieldrin) of 61.4% to 75.6% of the originally applied aldrin. The smallest amount of dieldrin was formed in the Plainfield sand, more in the muck soil, and the largest amount in the Carrington loam, though the total amount

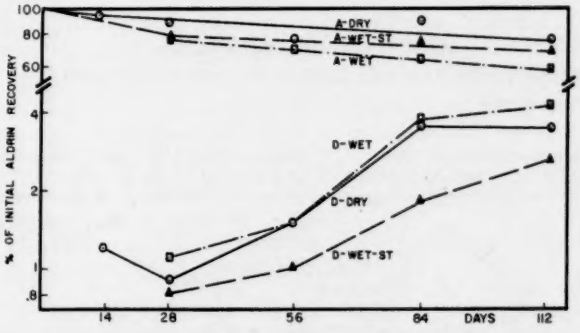

FIG. 2.—Conversion of aldrin (A) to dieldrin (D) in a Plainfield sand at 37° C.
Applied: aldrin 10 p.p.m.
WET = wet, nonautoclaved soil
WET-ST = autoclaved wet soil
DRY = nonautoclaved dry soil

Table 4.—Residues of aldrin and dieldrin found in a Quartz sand and three soil types 56 days after treatment (10 p.p.m.) with aldrin.[a]

Soil Type	Organic Matter (%)	In Per Cent of Initial Aldrin Recoveries		
		Total	Aldrin	Dieldrin
Quartz sand	0.0	1.2	0.7±0.3	0.5±0.1
Plainfield sand	0.8	61.4	57.9±0.7	3.5±0.0
Carrington loam	4.1	70.2	55.0±0.8	15.2±0.5
Muck	40.0	75.6	65.1±1.4	10.5±0.2

[a] Temperature, 37° C.

of toxicants recovered was slightly higher in the muck as compared with the loam soil. All these experiments were done in cotton plugged 500-ml. flasks. However, when the same experiment was carried out in open flasks, the amount of aldrin present in the Plainfield sand 56 days after treatment was slightly smaller than the amount found in the loam soil.

e) *The Persistence of Lindane in a Miami Silt Loam.*—After lindane had been applied to a Miami silt loam (organic matter content = 3.5%), it was found that lindane was most persistent in the dry soil and least in the wet nonautoclaved soil (fig. 3).

2) Field Experiments.—The analytical data (average of two replicated plots) obtained from the aldrin- and heptachlor-treated field plots are summarized in figure 4 and table 5. The nature of the residue curves was similar in the laboratory and field experiments despite the temperature and other environmental differences. The amount of aldrin recovered 17 months after field treatment was only 11.5% of the applied dosage, whereas dieldrin was recovered to an extent of 15.2% of the applied aldrin. The amounts of these two insecticides were equal 16 months after field treatment, as compared with 3.3 months under laboratory conditions at 37° C. (fig. 1).

Heptachlor was again found to be more persistent than aldrin, but the amount of dieldrin formed was nearly three times larger than the amount of heptachlorepoxide recovered. Yet, the total amounts of toxicants (aldrin +dieldrin or heptachlor+heptachlorepoxide) were very similar.

Paper chromatograms obtained from extracts of field soils treated with heptachlor revealed an additional spot that was located between the two obtained from reference grade heptachlor and heptachlorepoxide. A paper chromatogram prepared from the heptachlor emulsion which was used for field soil treatment revealed a similar spot in addition to the one obtained from heptachlor. In laboratory experiments in which analytical grade heptachlor (chromatographing as a single component) was used to treat soils, only heptachlor and its epoxide were recovered by paper chromatography.

Table 5.—Recoveries in p.p.m. of insecticidal residues from a Carrington loam 17 months after field treatment with 1) aldrin and 2) heptachlor at a rate of 25 lb./5″ acre.

			Per Cent of Total	
1) Aldrin	Dieldrin	A[a]+D[b]	A	D
1.84±0.32	2.45±0.19	4.29	43	57
			Per Cent of Total	
2) Hepta-chlor	Heptachlor-epoxide	H[c]+HO[d]	H	HO
3.70±0.05	0.94±0.10	4.64	80	20

[a] Aldrin. [b] Dieldrin. [c] Heptachlor. [d] Heptachlorepoxide.

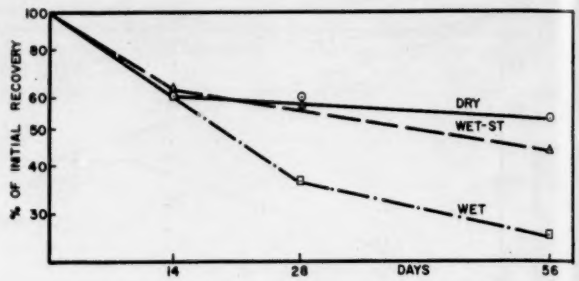

Fig. 3.—Recoveries of lindane residues (bioassay) from a Miami silt loam at 46° C.
Applied: lindane 10 p.p.m.
WET = wet, nonautoclaved soil
WET-ST = autoclaved wet soil
DRY = nonautoclaved dry soil

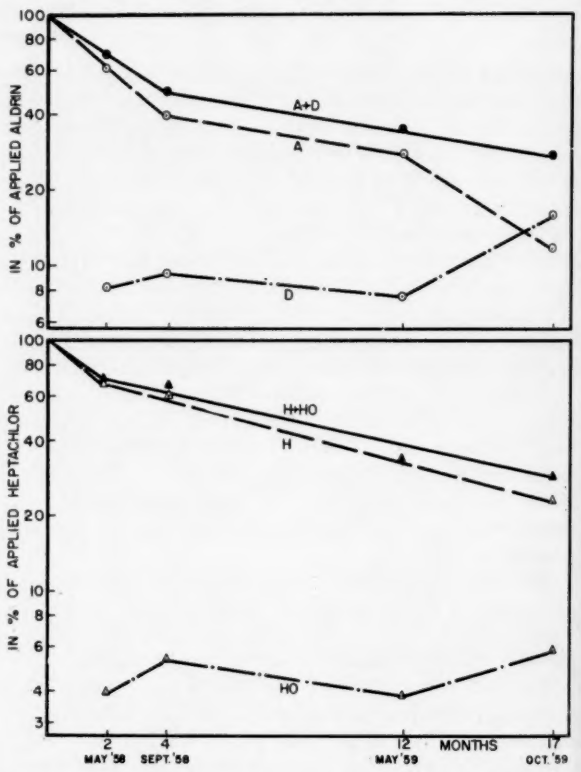

Fig. 4.—Recoveries of aldrin (A), dieldrin (D), heptachlor (H) and heptachlorepoxide (HO) from a Carrington loam. Treatment: aldrin or heptachlor 25 lb./5″ acre. Field experiment.

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The Use of Phorate to Control Aphids and the Hessian Fly on Winter Wheat[1]

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ABSTRACT

Field experiments were conducted from 1957 through 1959 to determine the effect of phorate in controlling insects on wheat. A randomized block design with all treatments replicated four times was used. Plots were seeded with a grain drill and harvested with a combine. Few insects infested the plots in 1957, but in 1958, heavy infestations of the hessian fly, *Phytophaga destructor* (Say), the apple grain aphid, *Rhopalosiphum fitchii* (Sand.), and the English grain aphid, *Macrosiphum granarium* (Kby.), were encountered. Phorate was applied as a seed treatment to control these insects in the fall. To control the spring brood of hessian fly and also aphids that might infest the plots, granulated phorate was broadcast in early April. Experiments were also conducted to determine the effect of the seed treatment under simulated conditions of grazing by clipping.

The phorate seed treatments caused significant losses in stand through reduced germination. However, such losses were not reflected in reduced yields. Both hessian fly and the aphids were controlled with a seed treatment of 0.5 lb. of phorate per 100 lbs. of seed. The spring brood of hessian fly was controlled by granulated 10% phorate broadcast at the rate of 1.75 lbs. of toxicant per acre. Highly significant increases in yield were obtained with all treatments where insects were controlled. All plots clipped to simulate fall grazing yielded from 4 to 6 bushels more per acre regardless of treatment.

Several investigators including Brown (1957, 1959), Guyer *et al.* (1958), Bigger (1959), and Wilson (1959) have reported control of the hessian fly, *Phytophaga destructor* (Say) with systemic insecticides, particularly phorate, applied either as a seed treatment or as granules in the row at time of seeding. Because of the promising results of these early observations, a study was initiated in Indiana with emphasis on the use of phorate as a seed treatment. Because of simplicity of application and probable low cost to the farmer, this method appeared to have certain advantages over granulated insecticides.

Experiments were designed with the following objectives: 1. To determine the most effective dosage for a seed treatment against the hessian fly and grain aphids in the field. 2. To determine the effectiveness of an application of a granulated material broadcast on the soil surface in the spring. 3. To determine the influence of insect control on yield. 4. To determine the effect of fall grazing on yield where wheat was seeded early and insects were controlled.

METHODS.—Studies were undertaken in 1957 and 1958 at various locations in the State. In all experiments a randomized block design was used, with treatments replicated four times. Plots were seeded in single drill widths $5\frac{1}{2}$ feet wide by 100 feet long. Yields were obtained by direct combining of each plot.

Three varieties of wheat were used: Knox, Vermillion, and Dual. The hessian fly resistant variety, Dual, was used to obtain comparisons in yield with early seeded susceptible varieties where the hessian fly was controlled. This method also made it possible to differentiate losses caused by grain aphids from those caused by the hessian fly. Varieties and dosage rates of phorate used are shown in tables 1 to 5.

All seed treatments were made by W. B. Gehl, of the American Cyanamid Company. The seed was first treated with a fungicide (captan). Phorate was then applied using a sticker consisting of 1.4 parts of rice oil, 2.0 parts sorbitol, and 0.6 parts of dextrin.

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Table 1.—The effect of phorate seed treatments on stands and yield of winter wheat seeded September 24, 1957, at Princeton, Indiana.

Variety	Pounds Phorate/ 100 Lb. Seed	Stand Count (Plants/ Ft.)	Yield (Bu./A.)
Dual	0	12.6	52.5
Vermillion	0	20.4	53.1
	0.25	10.6	49.1
	0.5	10.1	53.6
	1.0	9.8	50.9
L.S.D. at 5% level		3.2	1.6
at 1% level		4.5	3

Table 2.—The effect of phorate seed treatments on stand, insect populations, and yield of wheat varieties seeded Sept. 7, 1958 at Herbert Davis farm, Farmland, Indiana.

Variety	Pounds Phorate/100 Lb. Seed	Wheat Stand		Hessian Fly Infested (%)	Apple Grain Aphids/ Plant	Yield (Bu./ A.)
		Plants/ Linear Ft.	Loss (%)			
Vermillion	0	15.2	0	74.5	4.9	31.2
	0.5	14.5	5	6.5	0.0	39.0
	0.75	14.0	8	0.5	0.1	40.3
Knox	0	14.0	0	62.0	2.7	41.1
	0.5	12.5	11	0.5	0.5	47.1
	0.75	12.2	13	0.0	0.0	48.8
Dual	0	13.0	0	0.0	5.2	49.4
	0.5	11.7	10	0.0	0.1	51.5
	0.75	10.0	23	0.0	0.0	51.3
L.S.D. at 5% level		1.4				3.3
at 1% level		1.9				4.4

Table 3.—Control of the hessian fly with fall seed treatments and spraying applications of granulated phorate, Herbert Davis Farm, Farmland, Indiana.

Variety	Phorate Treatment		Hessian Fly Infestation (%)		Yield (Bu./A.)
	Fall Seed, Lb./100 Lb. Seed	Spring Broadcast, Lb./A.	Fall	Spring	
Dual	0	0	0	14	49.1
	0	1.75	0	0	54.1
Vermillion	0	0	74	56	31.5
	0	1.75	74	4	39.5
	0.5	0	7	24	39.0
	0.5	1.75	7	6	46.1
	0.75	0	1	34	40.9
	0.75	1.75	1	0	46.2
L.S.D. at 5% level					2.2
at 1% level					3.0

In all experiments, stand counts were made in all plots prior to any appreciable amount of tillering. Plant vigor was determined by rating each plot with a scale of 1–9. A rating of 1–3 indicated superior vigor, 4–6 average, and 7–9 low vigor.

RESULTS AND DISCUSSION.—*Germination.*—Data showed that treatment of wheat seed with phorate may cause a significant reduction in germination. This fact was evident in both 1957 and 1958 experiments (tables 1 to 3). Stand losses shown in table 4 were caused by infestation by the apple grain aphid, *Rhopalosiphum fitchii* (Sand.), and English grain aphid, *Macrosiphum granarium* (Kby.).

Table 4.—Control of the apple grain aphid on seedling wheat with a seed treatment of phorate, seeded at the Pinney-Purdue Farm, September 7, 1958.

Variety	Pounds Phorate/ 100 Lb. Seed	Stand Count Plants/ Foot 10/13/58	Plant Vigor[a] 11/12/58	Aphids/ Stem 11/12/58	Yield (Bu./A.)
Dual	0	6.6	6.0	12.3	39.1
Vermillion	0	7.0	5.0	9.9	35.2
	0.5	9.6	2.0	1.4	45.0
	0.75	12.0	1.0	2.2	44.4
L.S.D. at 5% level		0.7			3.5
at 1% level		1.0			n.s.

[a] Vigor estimated by using a 1–9 scale: 1–3 superior, 4–6 average, and 7–9 low.

Table 5.—Effect of fall clipping (to simulate grazing) on yields of wheat seeded in 1958 at the Herbert Davis Farm, Farmland, Indiana.

Variety	Pounds Phorate/ 100 Lb. Seed	Cultural Treatment	Yield (Bu./A.)	Insects Causing Losses
Vermillion	0	none	31.2	aphids, hessian fly
	0	clipped	37.8	aphids, hessian fly
	0.5	none	39.0	none
	0.5	clipped	45.9	none
	0.75	none	40.3	none
	0.75	clipped	46.9	none
Dual	0	none	49.4	aphids
	0	clipped	53.7	aphids
L.S.D. at 5% level			2.5	
at 1% level			3.4	

Combine harvested samples, however, indicated that no loss in yield resulted owing to the reduction in stand from phorate. In 1957 at Princeton, where stand loss was as high as 50%, yields were still high (table 1).

Hessian Fy Control.—The 1957 plots had no appreciable infestation of hessian fly. However, in the fall of 1958 untreated plots at the Herbert Davis Farm in Randolph County averaged as high as 75% of the plants infested. The following spring brood infested an average of 56% of the stems. Data in table 2 show that nearly complete control was obtained in the fall with seed treatments of both 0.5 and 0.75 lbs. of phorate per 100 lbs. of seed, whereas the spring brood (table 3) was controlled by an application of 1.75 lbs. of active granulated phorate broadcast on the field. Since the infestation in practically all phorate-treated plots was zero, statistical analysis was not applied to these data.

Aphid Control.—The same phorate-treated seed also controlled apple grain aphids and English grain aphids (tables 2 and 4).

Yield.—The most important effect of any treatment to a crop with an agricultural chemical is the influence it has on the yield. The data showed that highly significant increases in yield were obtained by using phorate on wheat. As a seed treatment, there was no significant difference between dosage rates of 0.5 and 0.75 lb. per 100 lbs. of seed. However, at the Herbert Davis Farm (table 2) this seed treatment resulted in an increased yield in the hessian fly susceptible varieties, Knox and Vermillion, ranging from 7 to 9 bushels per acre, while the hessian fly

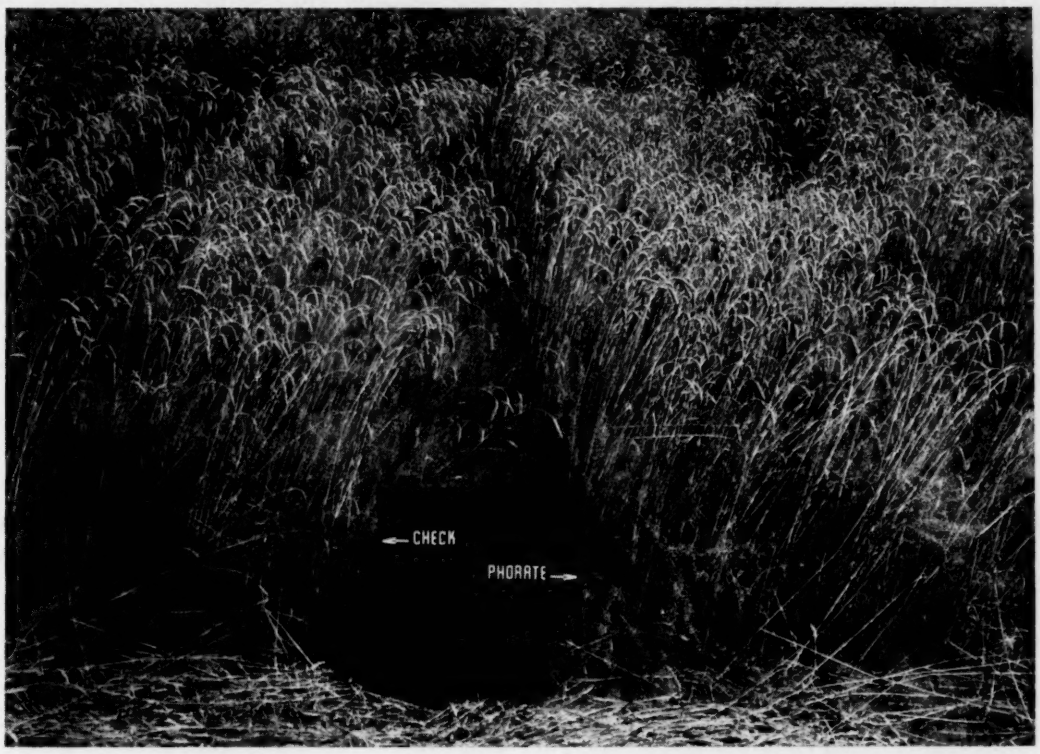

FIG. 1.—Wheat plots showing results of apple grain aphid control with phorate as a seed treatment.

resistant variety, Dual, had an average increase of 2 bushels. The latter apparently resulted from aphid control.

At the Pinney-Purdue Farm in Porter County, where no infestation by the hessian fly occurred, two species of aphids caused severe damage. Where these aphids were controlled, Vermillion averaged an increase of nearly 10 bushels of wheat per acre. In this experiment, where an untreated entry of Dual was included, it is interesting to note that Dual, under aphid infestation, yielded 6 bushels less than Vermillion, in which the aphids were controlled. In untreated plots of both varieties, Dual yielded more than Vermillion in the ratio normally expected.

A spring broadcast treatment with granulated phorate further increased yields (table 3). On Vermillion, the increase ranged from 5 to 8 bushels, while Dual had an increase of 5 bushels. Significant numbers of aphids were not observed in the experimental plots. However, within a period of a month between the final insect sampling and harvest, the plots may have been infested with the greenbug, *Toxoptera graminum* (Rond.), which was unusually prevalent in Indiana at that time. This condition may partially explain the increase of yield on Dual wheat. With Vermillion wheat, the combined fall seed treatment and spring broadcast application resulted in an increased yield of 14 bushels per acre.

An increased yield ranging from 4 to 6 bushels per acre was obtained in all cases by fall clipping (table 5) which is in accord with recent data by Hodges (1958) and Patterson *et al.* (1959). These data are of considerable significance to farmers who need to pasture wheat in the fall. Also, to provide fall forage, the crop must be seeded prior to the hessian fly-free date, which makes treatment for control a necessity with nonresistant varieties.

CONCLUSIONS.—Field experiments showed that the fall brood of the hessian fly and the apple grain and English grain aphids can be controlled on winter wheat with phorate applied to the seed at the rate of 0.5 lb. of toxicant per 100 lbs. of seed. Increase of the dosage did not increase the control significantly. In fact, laboratory studies to be reported in another paper suggest that the hessian fly may be controlled at a lower dosage than was used in these field studies. The spring brood of the hessian fly was controlled by a broadcast application of granulated 10% phorate applied at the rate of 1.75 lbs. of toxicant per acre in early April. Even though seed treatment with phorate reduced germination, highly significant increases in yields were obtained by controlling these insects. Fall seed treatments produced increased yields of 5 to 7 bushels per acre through hessian fly control and 2 to 10 bushels per acre by aphid control. The spring application of granules increased the yield by 4 to 6 bushels. At one location, combined fall seed and spring granule treatments on Vermillion wheat increased yields 14 bushels per acre. Experiments with fall clipping to simulate grazing, showed that regardless of insecticidal treatment, the clipped plots averaged 4 to 6 bushels more per acre.

From these data, it is concluded that yields of wheat can be greatly increased by controlling insects on early seeded wheat. Furthermore, the need for pasture and the favorable results obtained by fall clipping may lead to increased planting prior to the hessian fly-free date. This would make insect control mandatory on nonfly-resistant

varieties. However, results obtained with the variety Dual, indicated that treatment of a hessian fly-resistant variety to control aphids may be of considerably more significance than has been recognized in Indiana.

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Laboratory Selection of Normal and Chlordane-Resistant German Cockroaches for Resistance to Malathion and Diazinon[1]

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ABSTRACT

German cockroaches (*Blattella germanica* (L.)) from chlordane-resistant and normal strains were each placed under selection pressure to malathion and Diazinon®(*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) in the laboratory by exposing the nymphs to residual deposits of these materials on tempered Masonite and glass surfaces. The criterion of selection was >50% kill in the parental, F_1 and F_2 generations; >65% kill in the next two or three generations; and >80% kill thereafter.

Male and female cockroaches from all four selected strains were periodically tested for development of resistance, but data are presented herein for only the females. The following orders of resistance were obtained at LC-50 and LC-90, respectively: 6.9× and 1.5× in the normal strain selected to malathion when tested on filter paper, and 13.1× and 17.8× in the same strain when tested on panels; 9.4× and 1.4× in the chlordane-resistant strain selected to malathion when tested on filter paper, and 42.9× and 24.3× when tested on panels. Males and females in the chlordane-resistant strain were originally more susceptible to malathion than those in the normal strain, but development of resistance occurred more rapidly in the former, especially in the males, when it was placed under selection pressure to malathion.

Slight shifts toward steeper regression lines for Diazinon-selected strains occurred after six or seven generations, but the selected strains exhibited no detectable resistance to Diazinon.

Shifts in populations of the German cockroach (*Blattella germanica* (L.)) toward resistance following selection to DDT and BHC in the laboratory were shown as early as 1951 (Grayson 1951, 1953). Field strains resistant to chlordane probably existed as early as 1951 in Texas, but experimental evidence to confirm insecticidal resistance in a natural population was first published in 1953 (Heal *et al.* 1953). Later work confirmed resistance to chlordane, and showed that it extended to dieldrin, heptachlor, and lindane (Fisk & Isert 1953, Grayson 1954, Butts & Davidson 1955). Today resistance in the German cockroach is widespread over the United States, especially in the southern part, and it extends to many chlorinated hydrocarbon insecticides as well as to pyrethrins (Keller *et al.* 1956, Spear 1958).

Malathion and Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) have been used for some years in the control of German cockroaches resistant to chlorinated hydrocarbon insecticides. Many reports have been received of natural populations that have become difficult to control with one or the other of these materials. To the writer's knowledge, however, the only experimental evidence to indicate such resistance is that reported by Du Chanois as suspected resistance to malathion in German cockroaches collected from the kitchen of a U. S. Navy vessel while it was berthed at a naval base in Florida (personal communication). Burden *et al.* (1959) obtained from 4- to 11-fold resistance to malathion after 5 to 10 generations of selection in the laboratory. Starting with a normal strain, these investigators exposed successive generations of nymphs in a wind tunnel to contact sprays of malathion in deodorized kerosene, adjusted in concentration to give from 30% to 60% mortality.

In the study reported here, German cockroaches from chlordane-resistant and normal strains were each placed under selection pressure to malathion and Diazinon. The results after six or seven generations of selection are reported.

SELECTION PROCEDURE.—Nymphs from one-half to three-fourths grown were placed on tempered Masonite panels in a 1-gallon, glass battery jar in which both the

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panels and the inside of the jar contained residual deposits of the toxicant. The desired concentration of either malathion or Diazinon was prepared by water extension from the emulsifiable concentrate, and the panels were dipped in the emulsion in the battery jar. The length of time the nymphs were exposed to the treated surfaces, and the concentration of toxicant, were varied in order to obtain the desired percentage kill. In the parental, F_1 and F_2 generations the criterion of selection was greater than 50% kill; in the F_3 and F_4 for the normal strain, and in the F_3, F_4 and F_5 generations for the chlordane-resistant strain it was greater than 65% kill; and thereafter it was greater than 80% kill.

TESTING PROCEDURE.—Both malathion- and Diazinon-selected strains were tested periodically by exposing adult males and females to the appropriate toxicant applied to No. 1 Whatman filter paper in a modified WHO adult mosquito test apparatus. A predetermined amount of the toxicant as technical grade was added to 2 ml. of a 1:2 mixture of Risella oil-trichloroethylene and equally distributed over 200 sq. cm. of filter paper. The amount of Risella oil and area of filter paper were kept constant, but the amount of toxicant varied as the concentration was increased. At lower concentrations the toxicant was diluted with trichloroethylene to enhance accuracy in making volumetric measurements. The cockroaches were exposed for 1 hour and then placed in recovery jars. Mortality counts were made at the end of 1 and 3 days following treatment. Dead and moribound cockroaches were combined in the mortality counts. The criterion for regarding a specimen as moribound was its inability to exhibit active locomotion.

The malathion-selected strain was further tested by exposing adult males and females to residual deposits of malathion on tempered Masonite panels in battery jars. Both panels and inside of the jars were treated with malathion emulsion at different concentrations. After an exposure period of 24 hours the cockroaches were removed to recovery jars and 1-day mortality readings made. Mortality readings were again made 48 hours later, or 3 days from beginning of exposure. The criterion of mortality was the same as for the filter-paper technique.

Regression lines were fitted to the points by the method of least squares (Bliss 1935). From four to six points were used to establish each line, and at each concentration the tests were usually replicated from three to six times. Subsamples were composed of approximately 10 adult cockroaches of each sex for testing on filter paper, and from 10 to 20 of each sex for testing on tempered Masonite panels.

Table 1.—Resistance of female German cockroaches in malathion-selected strains.

STRAIN	METHOD OF TESTING	GENERATION	TIMES RESISTANCE LC-50	TIMES RESISTANCE LC-90
Normal strain selected to Malathion	Filter-paper	F_4	3.9	1.5
		F_6	6.9	1.5
	Masonite panels	F_4	6.9	16.3
		F_6	13.1	17.8
Chlordane-resistant strain selected to malathion	Filter-paper	F_5	4.6	1.3
		F_7	9.4	1.4
	Masonite panels	F_5	37.1	—
		F_7	42.9	24.3

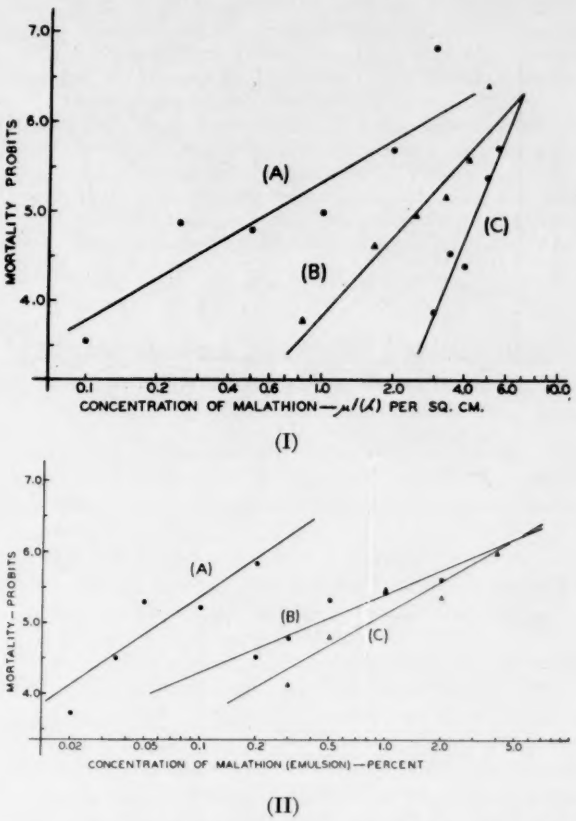
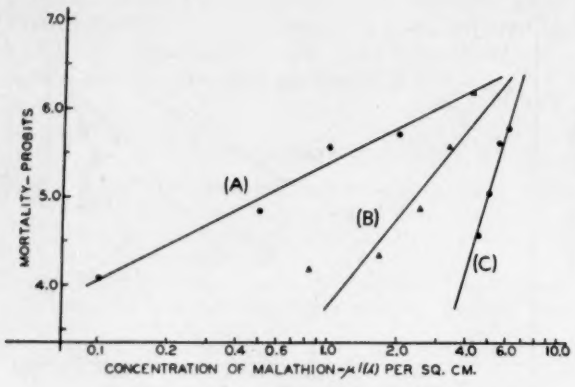


FIG. 1.—Mortality of female German cockroaches in normal and malathion-selected strains at the end of 3 days following: exposure for 1 hour to malathion on filter paper, (I); exposure for 24 hours to malathion on tempered Masonite panels, (II). Normal strain (A); normal strain selected to malathion for four generations, (B); same after six generations of selection (C).

RESULTS AND DISCUSSION.—The results are shown in figures 1 to 3 and in table 1. Dosage-mortality relationships in different generations of selection were obtained for both males and females but only those for the females are shown because the general pattern for the two sexes was similar. A striking observation at the beginning of the study was the greater susceptibility of the chlordane-resistant strain to malathion than that of the normal strain. LC-50 values for the males and females, respectively, were: 0.58 and 0.64 micro liters per sq. cm. for the normal strain, as compared with 0.24 and 0.52 for the chlordane-resistant strain, when tested on filter paper; and 0.07% and 0.062% for the normal strain, as compared with 0.03 and 0.035 for the chlordane-resistant strain, when tested on tempered Masonite panels. When selection pressure to malathion was applied, however, resistance developed more rapidly in the strain of chlordane-resistant origin. Also, as selection progressed the LC-50 and LC-90 values for males and females in each strain either became more nearly equal or those for the males were higher than corresponding ones for the females. For example, after the chlordane-resistant strain had been



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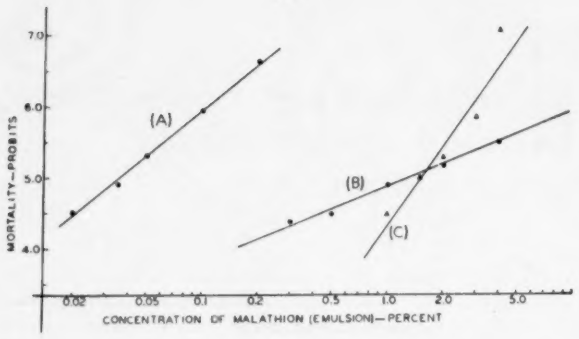

(II)

FIG. 2.—Mortality of female German cockroaches in chlordane-resistant and malathion-selected strains at the end of 3 days following: exposure for 1 hour to malathion on filter paper, (I); exposure for 24 hours to malathion on tempered Masonite panels (II). Chlordane-resistant strain (A); chlordane-resistant strain selected to malathion for five generations (B); same after seven generations of selection (C).

under selection pressure to malathion for seven generations the LC-50 values for males and females, respectively, were as follows: 4.9 and 4.9 microliters per sq. cm. when tested on filter paper, and 1.8% and 1.5% when tested on Masonite panels; similarly, the LC-90 values were 7.4 and 6.6 microliters per sq. cm. when tested on filter paper, and 5.0% and 3.4% when tested on Masonite panels.

Only slight differences in LC-50 and LC-90 values between males and females were observed in the initial response of normal and chlordane-resistant strains to treatment with Diazinon on filter paper. These differences showed a tendency to become even smaller as selection progressed, so that the males and females were almost equally susceptible to Diazinon.

When tested on filter paper a definite increase in slope of the regression lines for all selected strains was apparent. This increase did not occur in the early generations of selection when tested on tempered Masonite panels, but as selection progressed the regression lines became steeper. If the normal response of the German cockroach to selection with organic phosphate insecticides should be the rapid development of more homogeneous populations with steeper regression lines than in the original strain, this would be in sharp contrast to the response from selection with chlorinated hydrocarbon insecticides, which typically involves a long selection period with heterogeneous populations before the regression lines again become steep (Hoskins & Gordon 1956).

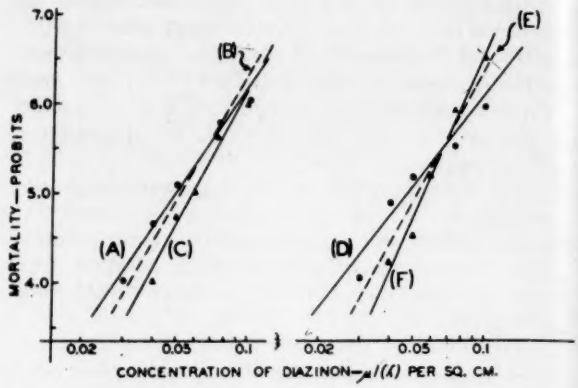

FIG. 3.—Mortality of female German cockroaches in normal, chlordane-resistant, and Diazinon-selected strains at the end of 3 days following exposure for 1 hour to Diazinon on filter paper. Normal strain (A); normal strain selected to Diazinon for four generations (B); same after six generations of selection (C); chlordane-resistant strain (D); chlordane-resistant strain selected to Diazinon for five generations (E); same after seven generations of selection (F).

The differences in results between the filter-paper and panel-testing procedures should be noted. These could be real differences, which will be repeated as selection continues. However, it is the writer's opinion that they may be attributed, at least in part, to the difference in length of exposure to the toxicant. The 1 hour of exposure to malathion on filter paper, as contrasted to 24 hours of exposure on panels, may have been an insufficient challenge to bring out the real difference between strains. Difficulty was experienced in getting the filter paper to retain a concentration of malathion sufficient to give high mortality in the strains of highest selection. It is therefore quite evident that the time of exposure must be increased, or some other modification made, if this technique is to be further used.

Selection to Diazinon apparently has produced little more than slight shifts toward more homogeneous populations. This result might be interpreted as slight "vigor tolerance," but certainly no real resistance is indicated. It will not be surprising, however, if resistance begins to appear after further selection because of the necessity in the F_8 and F_9 generations to increase the concentration of Diazinon for use in obtaining the desired kill of nymphs.

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Field Control of Cotton Mites with Aramite Aerial Spray in California[1]

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ABSTRACT

A phytotoxicity study with six potential spray oils was conducted on young Acala 4–42 cotton plants. Mobil GPD 1442 was found to be nonphytotoxic under the conditions of the test. Two emulsion concentrates and one oil solution formulation of Aramite® (2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite) were tested for phytotoxic reaction to field cotton. One emulsion concentrate formulation, containing 8 pounds of Aramite per gallon, was found to be of a low order of phytotoxicity under the conditions of the test. This formulation was field-tested applying 1.5 pounds of Aramite in 10 to 15 gallons of water per acre by Stearman bi-plane aircraft. Three species of phytophagous mites on cotton were controlled in all five field test locations.

Aramite® (2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite) has been successfully used for several years to control phytophagous mites on cotton in California (Smith & Bryan 1951, Hazel 1959). Most of the treatments have been made with 3% and 4% dusts applied by aircraft. The majority of aerial insecticidal and acaricidal applications on California cotton in past years have been in dust form. More recently, a strong trend toward aerial spray applications has been evident. This trend is the result of several factors. Ranking high among these factors is the visible undesirable drift of dust to adjacent crops that do not have a residue tolerance for the pesticide. These circumstances made it desirable to develop and field test an aerial spray formulation of Aramite on California cotton.

The commercial use of Aramite on most crops has been largely limited to dust and water suspension type formulations. Oil in water emulsion type formulations are frequently phytotoxic (Clower & Matthysse 1954). The first phase of this study was to select a potential nonphytotoxic spray oil carrier for Aramite. The second phase was to select a low-phytotoxic Aramite emulsion concentrate formulation. The third phase was to field test the least phytotoxic formulation on phytophagous mites infesting cotton with comparison with other acaracidal sprays.

OIL PHYTOTOXICITY TESTS.—Acala 4-42 cotton plants were grown to the four to six true-leaf stage in individual containers. Ten plants per treatment were placed in a row on the ground with a spacing of 1 foot between plants. Oil treatments were made with a garden tractor-mounted power sprayer. Two nozzles were used applying the oils at a rate of 2 gallons per acre with 30 p.s.i. pressure. All plants were covered to the point of run-off. The plants were then placed in a greenhouse and held at a temperature of 90° to 110° F.

Apparent phytotoxic effect was observed at several intervals. The results shown in table 1 were obtained after 7 days. The phytotoxic rating system used is similar to that described by Clower & Matthysse (1954). The ratings of individual plants are given to show the variation that occurs within a group of 10 cotton plants.

An analysis of variance[4] of the data presented in table 1 yielded a significant difference between treatments at the 0.0005 level. At the 0.05 level Treatment No. 2 is significantly more phytotoxic than any of the other treatments; Treatment No. 6 is significantly more phytotoxic than No. 4 and No. 7; and Treatments No. 1, 3, 4, 5, and 7 are not significantly different from each other.

FORMULATION PHYTOTOXICITY TEST.—Two emulsion concentrates and one oil solution formulation of Aramite were field tested on Acala 4-42 cotton in July, 1959. The emulsion concentrate formulations tested were:

Formulation	*Ingredient*	*Per Cent by Weight*
6 lb./gal.	Technical Aramite (90%)	75.0
	Xylene	20.0
	Triton X-151	2.5
	Triton X-171	2.5
	Total	100.0
8 lb./gal.	Technical Aramite (90%)	94.0
	Emulphor EL719	6.0
	Total	100.0

The oil solution was made by mixing the required amount of technical Aramite with Mobil GPD 1442.

These formulations were applied to cotton which was 18 to 24 inches in height. All materials were applied at a rate of 1.5 pounds of Aramite per acre. The emulsions were ap-

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[3] Entomologist, A. F. C., Incorporated, Edison, California.

[4] The authors gratefully acknowledge the statistical analysis of the data by Mr. Reed Smith, Research and Development Department, United States Rubber Company, Naugatuck Chemical Division.

Table 1.—Phytotoxicity[a] series to oils. June, 1959.

Treatment No.		Individual Cotton Plants										Average
		1	2	3	4	5	6	7	8	9	10	
1	Standard—Pacific Base Oil 50 Pale	0	1	2	0	0	0	0	0	0	2	0.5
2	Standard—Chevron Paraffin Oil	7	6	8	4	4	4	8	6	9	5	6.1
3	Union—No. 90 Neutral Oil	0	1	1	1	1	0	0	1	1	0	0.6
4	Mobil—GPD 1442	0	0	0	0	0	0	0	0	0	0	0.0
5	Mobil—GPD 1432	0	2	1	0	0	0	0	0	0	0	0.3
6	Phillips—Base Oil No. 2	0	0	7	10	1	0	1	2	0	0	2.1
7	Untreated	0	0	0	0	0	0	0	0	0	0	0.0

[a] Index: 0, None; 1, slight; 4, moderate; 8, severe; 10, kill.

plied in 10 gallons of water per acre with 20 ounces of Colloidal Products Multifilm X-77 per 100 gallons of water added as a spreader. The oil solution was applied at 2 gallons per acre. The results of phytotoxic ratings after 1 week are shown in table 2.

An analysis of variance of the data presented in table 2 yielded a significant difference between treatments at the 0.0005 level. At the 0.05 level treatments No. 1 and No. 3 are significantly more phytotoxic than No. 2 and No. 4; and Treatment No. 2 is not significantly different from No. 4.

The type of coverage obtained with a ground spray application is probably different from that obtained with an airplane spray application. Therefore, about 5 acres of healthy cotton was treated with the same oil formulation, rate, and volume by a Stearman biplane. Within 12 hours after treatment 30% to 50% of the cotton foliage was covered with necrotic spots.

Field Tests.—The Aramite (8 lb. per gal.) emulsion was field tested in five locations of the Southern San Joaquin Valley of California. Direct comparisons were made with Tedion® (2,4,4′,5-tetrachlorodiphenyl sulphone) emulsion in two tests and with Kelthane® (1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol) emulsion in one test. All fields were infested with a mite complex consisting of: two-spotted spider mites (*Tetranychus telarius* (L.)), strawberry spider mites (*T. atlanticus* McG.), and Pacific spider mites (*T. pacificus* McG.)

Table 2.—Phytotoxic[a] effect of several Aramite[b] formulations on cotton, July and August, 1959.

Treatment No.	Formulation	Treatment			Phytotoxicity			
		Water	Oil	Total Gal. per A.	Replicate 1	2	3	Average
1	6 lb./gal. emulsion	X		10	3	3	4	3.3
2	8 lb./gal. emulsion	X		10	1	1	2	1.3
3	Oil solution		X	2	4	6	5	5.0
4	Untreated	X		10	0	0	0	0.0

[a] Index: 0, none; 1, slight; 4, moderate; 8, severe; 10, kill.
[b] Applied at 1½ gallons of toxicant per acre.

(Baker & Pritchard 1953). The two-spotted spider mite was the predominate species in all of the fields. The treatments were made with three different Stearman biplane aircraft. Spray pattern and drift with aircraft applications are such that it is difficult to have an untreated check area adjacent to treatments. In these tests, nearby untreated mite-infested fields were located in each test area. The mite populations were carefully followed in these untreated fields and no indication of decline in populations was observed. Estimates of mite control were obtained by inspecting at least 100 leaves in each field prior to treatments and at intervals of 4, 7, 14, and 21 days after treatments. An estimated 90% or more control was considered to be good economic control. The results shown in table 3 indicate that the materials and treatments resulted in fair

Table 3.—Large scale field tests of mite control on cotton, August and September, 1959.

Plot	Acaracide	Acres	Pounds per Acre			Gal. per A.	X-77 per 100 Gal. Water (Oz.)	Estimated Per Cent Mite Control[a] After Days Shown			
			Acaracide	Toxaphene	DDT			4	7	14	21
1[b]	Aramite	14	1.5	0	0	15	10	87	95	97	98
2	Aramite	6	1.5	2	1	15	10	85	95	98	99
	Tedion	12	0.5	2	1	15	10	25	75	80	99
3[c]	Aramite	12	1.5	2	1	10	10	70	90	96	98
4	Aramite	16	1.5	2	1	10	10	40	80	90	99
	Kelthane	16	1.1	2	1	10	10	40	50	75	86
	Tedion	16	0.5	2	1	10	10	30	60	90	98
5[d]	Aramite	40	1.5	0	1.5	15	10	90	98	98	99

[a] Based on observations of at least 100 leaves per treatment.
[b] Two rows of cotton between each row of small peach trees.
[c] Four rows of cotton between each row of grapes.
[d] Very heavy mite population—25 to 50 per leaf—leaves discolored.

to good economic control. No evidence of phytotoxic effect could be found in any of the treatments.

The cotton plants at 4 of the 5 locations were 5 to 7 feet in height and frequently lodged. It seems unlikely that good under-leaf coverage of the spray occurred. However, careful inspection of heavily infested leaves deep in the foliage canopy invariably showed excellent mite control with both Tedion and Aramite. This may suggest control by means of subcuticular residues as noted by Ebeling & Pence (1954).

Numerous unidentified predacious mites were present on the cotton at two locations. These predators did not appear to be greatly affected by the Aramite treatments. This phenomena was previously noted by Huffaker & Kennett (1956).

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Stability, Toxicity, and Reaction Mechanism with Esterases of Certain Carbamate Insecticides[1]

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ABSTRACT

The stability, activity as inhibitors of acetyl- and butyrylcholinesterase, and toxicity to house flies, *Musca domestica* L., and mice were compared for 30 *N*-alkyl and *N,N*-dialkylcarbamates. For each type of biological activity a distinctive structural specificity unrelated to stability was observed. The carbamates varied greatly in their inhibitory specificity for the acetyl- and butyrylcholinesterase and in the rate of attaining an equilibrium of cholinesterase, carbamate and acetyl choline during assay. Plasma albumin was found to hydrolyze *p*-Nitrophenyl *N*-alkylcarbamates rather than plasma cholinesterase or arylesterase. Chymotrypsin and human plasma butyrylcholinesterase did not react with the carbamates in a similar manner to their reaction with the acetates, ethyl carbonates and dialkyl phosphates derived from the same enols. These results indicate that competition rather than carbamoylation is the primary mechanism of cholinesterase inhibition and presumably toxicity for the *N*-alkyl and *N,N*-dialkylcarbamates studied.

The usefulness of N-methyl and N,N-dimethylcarbamates as insecticides is well established. Among the active compounds are Sevin® (1-naphthyl *N*-methyl carbamate), Isolan® (1-isopropyl-3-methyl-5-pyrazolyl dimethylcarbamate), Pyrolan® (1-phenyl-3-methyl-5-pyrazolyl dimethylcarbamate), Pyramat® (2-n-propyl-4-methyl-6-pyrimidyl dimethylcarbamate), and dimetan (5,5-dimethyldihydro resorcinyl dimethylcarbamate) (Gysin 1952, Haynes *et al.* 1957). Certain other N-alkyl and N,N-dialkylcarbamates are also used clinically because of their miotic action upon the pupil, ability to increase the tone and motility of the intestine, anticurare action and activity in restoring muscle strength in myasthenia gravis (Stempel & Aeschlimann 1956). Each of these actions of the carbamates appears to be due at least in part to their powerful anticholinesterase activity. A quarternary nitrogen structure in the molecule usually increases the pharmacological activity of these compounds in mammals (Stempel & Aeschlimann 1956), but decreases their efficiency as insecticides (Gysin 1954, Kolbezen *et al.* 1954) because of an inability of the ionized molecules to penetrate insect cuticle and fatty nerve sheath (Kolbezen *et al.* 1954, O'Brien 1957). The anticholinesterase activity and toxicity to certain insects of the substituted-phenyl N-methylcarbamates are inversely dependent on the rates of hydrolysis of the carbamates (Kolbezen *et al.* 1954). Cholinesterases (Goldstein & Hamlisch 1952, Myers 1952, 1956), plasma albumin (Casida & Augustinsson 1959), and an enzyme(s) in rabbit plasma which also hydrolyzes di*iso*propyl phosphoryl fluoride (Augustinsson & Casida 1959) have been implicated as proteins active in the decomposition of some carbamate esters.

An attempt was made to compare the mechanism for the reaction of aryl acetates, ethyl carbonates, diethyl phosphates, and carbamates with the esteratic site of several proteins. Further, the stability, anticholinesterase activity, and toxicity to mice and house flies (*Musca domestica* L.) were determined for several carbamate insecticides and their less toxic analogs.

MATERIALS.—*Carbamates.*—The carbamates studied (table 1) were selected on the basis of high insecticidal activity and/or available analytical methods to follow their hydrolysis and were restricted to compounds that would not exist as ionized molecules either under physiological conditions or at high hydroxyl ion concentrations used in stability determinations. Included among the carbamates utilized were Sevin (compound 17, table 1), Isolan (compound 21), Pyrolan (compound 22) and dimetan (compound 24). Compounds 1–13 and 16–28 were provided by Dr. H. Gysin of J. R. Geigy, Basle,

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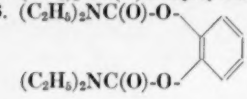
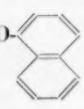
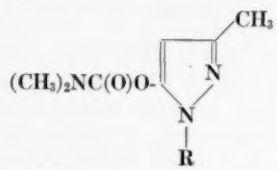
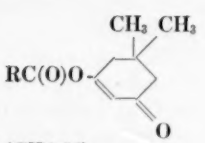
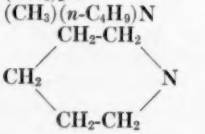
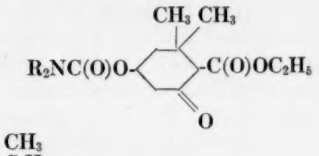
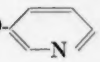
Table 1.—Stability, anticholinesterase activity and toxicity of certain N-alkyl and N,N-dialkylcarbamates.

Carbamate	K(OH) 1. Mole^{-1}Min.$^{-1}$	pI_{50} cholinesterase (or % Inhib. at 10^{-3} M)		LD_{50} (Mg./Kg.)	
		Torpedo elec. org.	Human Serum	Mouse *i-p*	House Fly Topical
PHENYL					
$RHNC(O)O\text{-}C_6H_4\text{-}NO_2\text{-}p$					
1. CH_3	1.3×10^{5}	4.3	4.5	35	>2,000
2. C_2H_5	1.6×10^{5}	3.8	4.0	25	>2,000
3. $C_3H_7\text{-}n$	1.6×10^{5}	4.4	4.3	30	>2,000
4. $C_3H_7\text{-}i$	1.9×10^{5}	3.1	6.3	25	>2,000
$R_2NC(O)O\text{-}C_6H_4\text{-}NO_2\text{-}p$					
5. CH_3	2.0×10^{-2}	(46%)	3.7	125	>2,000
6. C_2H_5	3.6×10^{-4}	(38%)	(45%)	155	1,000–2,000
7. $C_3H_7\text{-}n$	1.7×10^{-4}	(47%)	4.4	145	>2,000
8. $C_3H_7\text{-}i$	5.1×10^{-6}	(0%)	(40%)	130	>2,000
9. $C_4H_9\text{-}n$	2.5×10^{-5}	(20%)	4.2	160	>2,000
10. $(CH_3)_2NC(O)O\text{-}C_6H_4\text{-}NO_2\text{-}o$	4.6×10^{-3}	5.1	5.8	3	100
11. $(CH_3)_2NC(O)O\text{-}C_6H_5$	3.9×10^{-3}	(10%)	4.4	250	450
12. $(C_2H_5)_2NC(O)O\text{-}C_6H_5$	6.2×10^{-5}	(0%)	3.2	200	1,000
13. $(C_2H_5)_2NC(O)CH_2O\text{-}C_6H_5$	[a]	(0%)	3.2	500	>2,000
14. $(CH_3)_2NC(O)O\text{-}C_6H_4\text{-}NH_2\text{-}m$	1.7×10^{-3}	(36%)	4.6	100	>2,000
15. $(CH_3)_2NC(O)O\text{-}C_6H_4\text{-}CH_2N(CH_3)_2\text{-}o$	1.0×10^{-3}	6.8	6.6	0.3	250
16. 	$<5\times10^{-5}$	(4%)	3.4	70	>2,000
1-NAPHTHYL 					
17. H[b]	7.7×10^{1}	6.7	5.4	31	250, 75[c]
18. CH_3	1.2×10^{-3}	6.0	6.3	62	700
3-METHYL-5-PYRAZOLYL 					
19. H	4.1×10^{-3}	3.8–4.2	6.6	13	>2,000
20. C_2H_5	8.3×10^{-2}	5–6	6.9	0.75	25
21. $C_3H_7\text{-}i$[b]	6.9×10^{-2}	5.5–6.5	7.7	0.75	25, 15[c]
22. C_6H_5[b]	6.2×10^{-2}	4.5–5.5	7.8	5	90
23. $C(O)N(CH_3)_2$	2.0×10^{-3}	4–5	6.3	3	2
5,5-DIMETHYLDIHYDRORESORCINYL 					
24. $(CH_3)_2N$[b]	2.0×10^{-1}	3.5–4	6.2	33	160
25. $(CH_3)(n\text{-}C_4H_9)N$	4.0×10^{-2}	(37%)	6.2	125	>2,000
26. 	2.2×10^{-1}	(20%)	7.8	150	>2,000

[a] The phenoxy ether bond was not cleaved to yield phenol in 1 *M*. sodium hydroxide.
[b] Trademark or common names for insecticides: Sevin (17), Isolan (21), Pyrolan (22) and dimetan (24).
[c] Toxicity data by comparable technique (Mengle & Casida 1958).

Table 1.—(Continued)

CARBAMATE	K(OH) 1. MOLE^{-1}MIN.$^{-1}$	pI_{50} CHOLINESTERASE (OR % INHIB. AT 10^{-3} M)		LD_{50} (MG./KG.)	
		Torpedo elec. org.	Human Serum	Mouse *i-p*	House Fly Topical
5,5-DIMETHYL-4-CARBETHOXY-CYCLOHEXAN-3-ON-1-YL 					
27. CH_3	2.2×10^{-1}	4–5	7.2	20	100
28. C_2H_5	1.7×10^{-2}	(12%)	5.7	100	>2,000
3-PYRIDYL 29. $(CH_3)_2NC(O)O$- 	2.3×10^{-2}	(45%)	5.0	10	800
FLUORO 30. $(CH_3)_2NC(O)F$	>1[d]	3.5–4.5	5.5	0.17	45[c]

[d] Based on chromatography and loss of anticholinesterase activity (Augustinsson & Casida, 1959).

Switzerland; compound 14 by Dr. J. A. Aeschlimann of Hoffmann-La Roche Inc., Nutley, New Jersey, U.S.A.; compounds 15 and 29 by Dr. E. Silberschmidt of Hoffmann-La Roche, Inc., Basle, Switzerland; compound 30 by Dr. G. Schrader of Farbenfabriken Bayer, Wuppertal-Elberfeld, Germany; and a separate sample of compound 17 by Carbide Carbon Chemical Co. through the courtesy of Dr. L. E. Limpel, Boyce-Thompson Institute for Plant Research, Yonkers, New York, U.S.A. According to information from the suppliers, the compounds were of high purity. Analyses showed them to be free of hydrolysis products and that under suitable alkaline conditions the hydrolysis rates corresponded to single component samples.

The structures indicated for compounds 19 and 23 supplied by Geigy were further studied because of the great selective toxicity difference between these two pyrazolyl carbamates. Elemental analyses (carbon, hydrogen, and nitrogen), infrared spectra and the intensity and extractability into chloroform of the dyes formed from the reaction of their hydrolysis products with 4-aminoantipyrine were consistent with the structures designated.

Enzyme Preparations.—The various fractions of human postpartum serum studied were those obtained by a partly modified method No. 6 of Cohn *et al.* (1950). These fractions were prepared and provided by A/B KABI (Stockholm, Sweden). Chymotrypsin was a crystallized salt-free preparation from the Worthington Biochemical Co. (Freehold, New Jersey, U.S.A.). The acetylcholinesterase studied was a purified preparation from the electric organ of *Torpedo marmorata*.

METHODS.—*Analytical Methods.*—The hydrolysis of nitrophenyl esters was followed by measurement of the absorbance of the nitrophenate ion at 400 mμ on a Beckman Model DU spectrophotometer (Hartley & Kilby 1952, 1954).

For following the course of hydrolysis of carbamates derived from phenol, naphthol, and heterocyclic enols, the 4-aminoantipyrine method was utilized (Emerson 1943, Emerson & Beegle 1943, Casida & Augustinsson 1959). The colored dyes from all the enols except those containing a free carboxy group were completely extracted from water into an equal volume of chloroform. This observation was found useful in differentiation of the nature of the hydrolysis reaction in compounds 16, 23, 27 and 28 of table 1 where a free carboxylic group would be formed on hydrolysis at one of the possible sites.

Stability Tests.—For studies on enzymatic hydrolysis of the nitrophenyl esters (table 2), the esters were initially dissolved in ethanol containing the emulsifying agent, Tween 20, and then 0.05 *M* sodium phosphate buffer at pH 7.80 was immediately added to yield final concentrations of 7.5×10^{-5} *M* nitrophenyl ester, 0.2% ethanol and 0.02% Tween 20. All hydrolysis reactions at 22° C. with the nitrophenyl esters closely approximated linear relationships when the logarithm of the percentage of unhydrolyzed substrate was plotted against time of reaction. Results are therefore expressed as $K_{hyd.}$ (min^{-1}) obtained by dividing the half-life of the substrate in min into 0.693.

The second-order $K_{hyd.}$ at 22° C. was determined for each of the carbamic acid esters listed in table 1. These constants were determined and calculated by the general procedures outlined by Kolbezen *et al.* (1954). The stability of the *p*-nitrophenyl N-alkylcarbamates was determined in pH 7.80 sodium phosphate buffer as indicated above. Hydrolysis of the nitrophenyl N,N-dialkylcarbamates was followed by determining the rate of nitrophenate ion liberation from 7.5×10^{-5} *M* solutions in 1 *M* aqueous sodium hydroxide in the absence of Tween 20 The aminoantipyrine reagent was used with the phenyl

naphthyl and heterocyclic carbamates. The reaction mixture contained 1×10^{-3} *M* carbamate, 0.2% ethanol and 0.02% Tween 20. The compounds all appeared to be in solution except compound 17 which was present as a fine suspension. The sodium hydroxide concentration during hydrolysis was 0.01 *M* for compound 17 and 1 *M* for compounds 10 to 16 and 18 to 29. After the desired hydrolysis time for these carbamates in 1.0 ml. sodium hydroxide solution, 1.0 ml. of an equivalent molarity sodium dihydrogen phosphate solution was added and the mixture shaken. The color was then developed by adding 0.2 ml. of 0.2% aqueous 4-aminoantipyrine followed by 0.2 ml. of 0.8% aqueous potassium ferricyanide and the absorbance determined as previously described (Casida & Augustinsson 1959). The specific absorbance of the red dye varied with the enol and so 6 to 9 ml. water was used for dilution of the dye from the substituted phenyl and naphthyl compounds, and 3 to 5 ml. for the heterocyclic compounds. Zero time determinations were made by reversing the order of addition of the alkali and phosphate. Care was taken with the longer hydrolysis times to protect the sodium hydroxide solutions from carbon dioxide absorption during the hydrolysis period. All hydrolysis curves closely approximated a linear relationship when the logarithm of the percentage unhydrolyzed substrate was plotted against the time of reaction. The constants are expressed as K_{OH} (1. mole^{-1} min^{-1}), although the results for compounds 1 to 4 are not directly comparable with the others because of the possible catalytic effect of the phosphate on the ester hydrolysis (Casida & Augustinsson 1959).

Anticholinesterase Activity.—The inhibitory activity of the carbamic acid esters (table 1) was determined with purified cholinesterase preparations from human serum and the electric organ of *Torpedo marmorata*. The carbamate solutions were prepared in distilled water to which the enzyme and bicarbonate-CO_2 buffer (pH 7.6) were then immediately added to yield a total volume of 1.60 ml. in the flask. Precautions were taken (especially with the highly unstable compounds 1–4 of table 1) to bring the inhibitor in contact with the enzyme with minimum spontaneous hydrolysis before mixing. After 35 min. incubation of the inhibitor with the enzyme at 25° C., the acetylcholine substrate in 0.40 ml. was tipped from the side arm to yield a final concentration of 0.01 *M*. In the absence of inhibitor the CO_2 evolved in 30 min. from each flask was 135 μliters with 136 μg. protein for the human serum cholinesterase, and 150 μliters with 250 μg. electric organ preparation. When carbamates were present, the rate of CO_2-evolution at substrate-inhibitor-esterase equilibrium (Augustinsson & Nachmansohn, 1949) was used in determining the degree of inhibition. The efficiency of esterase inhibitors is expressed in pI_{50} values— *i.e.* the negative logarithm of molar carbamate concentration producing 50% inhibition at equilibrium of substrate and inhibitor. Where less than 50% inhibition resulted at 10^{-3} *M* carbamate, the observed per cent inhibition at this concentration is reported (table 1).

Toxicity Determinations.—Approximate LD_{50} values for the carbamates were determined by the topical route for house flies and by the intraperitoneal route for white mice. Female house flies were topically treated according to a described procedure (Mengle & Casida 1958) with acetone solutions of the carbamates and mortality counts taken 24 hours later. White mice of an inbred strain and mixed sex weighing about 20 gm. each were injected with propylene glycol solutions of the carbamates numbered 10 to 30. Mortality counts were taken 24 hours after injection of 0.10 ml. of the propylene glycol solutions per 10 gm. body weight. Compounds 1 and 6 to 9 were so low in solubility in propylene glycol that saturated solutions were not lethal. The more favorable solvent, glycerol formal, was therefore used for the *p*-nitrophenyl carbamates (compounds 1 to 9) with 0.10 ml. of solution per 20 gm. body weight and 25-gm. female Rolfsmeyer (Madison, Wisconsin) white mice. The LD_{50} values for mice listed in table 1 are approximations since only about 40 mice were used per compound.

RESULTS.—*Relation of Structure to Stability.*—Stability determinations for a series of carbamic acid esters in aqueous alkali (table 1) showed a great variation in stability with different molecular substituents. It appears likely that the alkaline hydrolysis of the N-alkylcarbamates yielded the enol, alkylamine and carbonate ion, possibly with the alkyl*iso*cyanate as an intermediate product according to Aeschlimann & Reinert (1931). The N,N-dialkylcarbamates probably yielded the enol, dialkylamine and carbonate ion (Michler & Escherich 1879, Hantzsch & Sauer 1898). The order of increasing stabilizing effect on the ester bond for the alkyl constituents in the carbamoyl group were as follows: mono-*iso*propyl < monoethyl < mono-*n*-propyl < monomethyl < pentamethylene or dimethyl < diethyl < di-*n*-propyl < di-*n*-butyl < di*iso*propyl. The effect of alkyl group, particularly *iso*propyl, was much different in the monoalkyl as compared with the dialkylcarbamoyl series.

The nature of the acid or enol also greatly affected the stability of the ester bond. With the N,N-dimethylcarbamates the order of increasing stability to alkaline hydrolysis was as follows:

fluoro < 5,5-dimethyl-4-carbethoxy-cyclohexan-3-on-1-yl < 5,5-dimethyldihydroresorcinyl < 1-*iso*propyl-3-methyl-5-pyrazolyl < 1-phenyl-3-methyl-5-pyrazolyl < 3-pyridyl < *p*-nitrophenyl < *o*-nitrophenyl < 3-methyl-5-pyrazolyl < phenyl < 1-(N,N-dimethylcarbamoyl)-3-methyl-5-pyrazolyl < *m*-aminophenyl < 1-naphthyl < *o*-dimethylaminomethylphenyl.

On hydrolysis of compounds 16 and 23 in 1 *M* sodium hydroxide, the cleavage occurred predominantly at a single N,N-dialkylcarbamoyl group since no products were formed which yielded chloroform-insoluble dyes on reaction with 4-aminoantipyrine. With compounds 27 and 28 the initial cleavage was at the N,N-dialkylcarbamoyl group since the initial hydrolysis products were completely chloroform-soluble. However, on prolonged standing of the products in alkali, the dye formed from these products became progressively less chloroform-soluble as if the 5,5-dimethyl-4-carbethoxy-cyclohexan-3-on-1-ol was subsequently cleaved at the carbethoxy group.

IN VITRO *Anticholinesterase Activity.*—The course of the enzymatic hydrolysis of acetylcholine in the presence of varying concentrations of the carbamates shown in table 1 was studied. When the enzyme and inhibitor

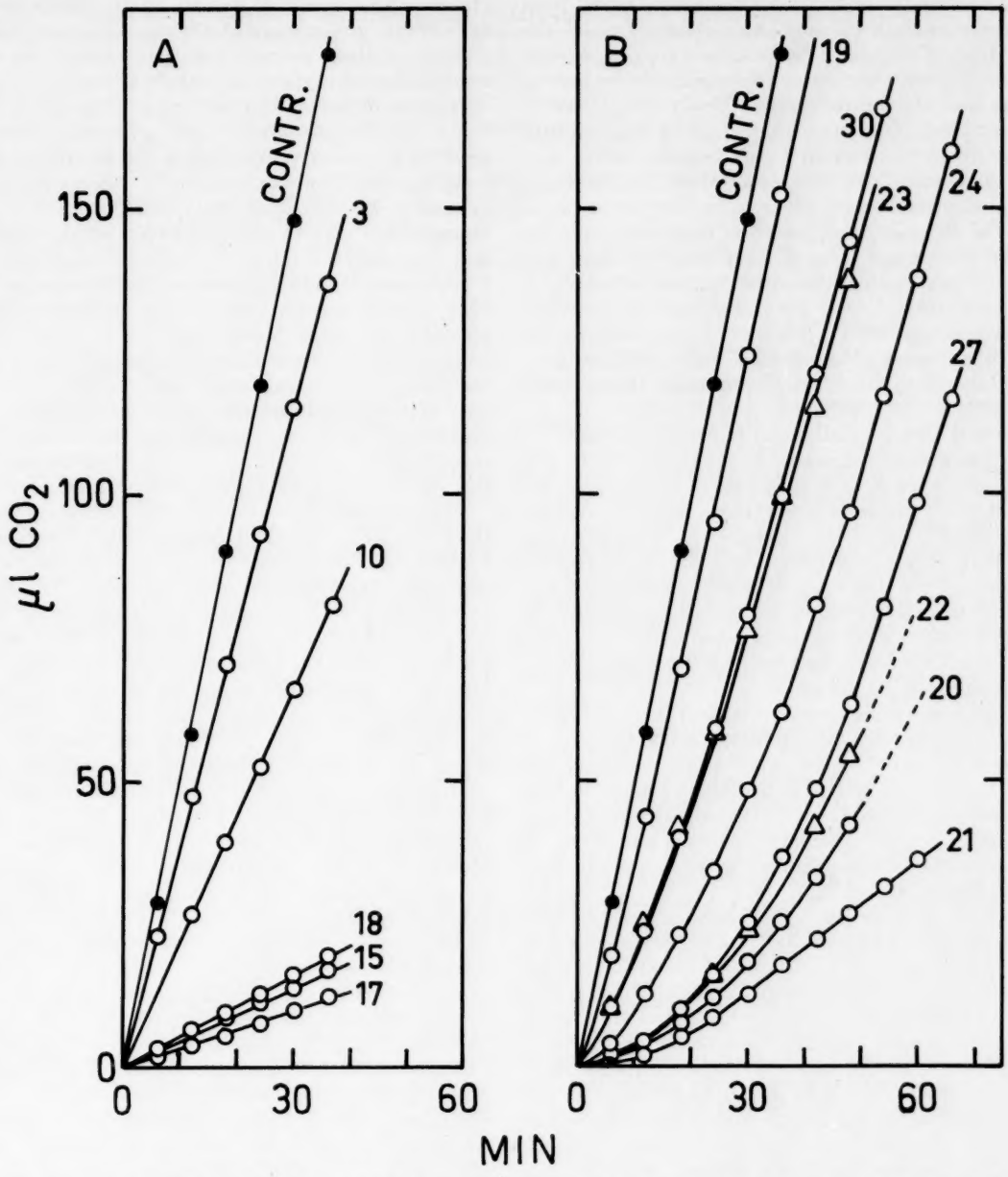


FIG. 1.—Course of enzymatic hydrolysis of acetylcholine by *Torpedo* acetylcholinesterase in the presence of various carbamates at 1×10^{-5} *M*. Numbers in the graph refer to compound designations in table 1. A: carbamates of aromatic enols; *B*: carbamates of heterocyclic enols and N,N-dimethylcarbamoyl fluoride.

were incubated together for 35 min. prior to adding the substrate, the subsequent time required for attaining an equilibrum of cholinesterase-acetylcholine-carbamate varied with the inhibitor and enzyme. The equilibrium with the human serum butyrylcholinesterase was reached very quickly with all the carbamates. However, several minutes were required after the addition of acetylcholine to achieve this equilibrium with the *Torpedo* acetylcholinesterase. A similar relationship has been reported for the acetylcholinesterase of *Electrophorus electricus* and physostigmine or prostigmine (Augustinsson & Nachmanson 1949). An equilibrium between *Torpedo* acetylcholinesterase, carbamate and acetylcholine was attained much more quickly with the aromatic carbamates (fig. 1 A) than with the heterocyclic or fluoro carbamates (fig. 1 B). The slow attainment of an equilibrium with the heterocyclic and fluoro carbamates necessitated reporting the pI_{50} values as a range rather than an absolute value with the *Torpedo* acetylcholinesterase (table 1).

Most of the carbamates studied were more active inhibitors for the human serum butyrylcholinesterase than for the *Torpedo* acetylcholinesterase (table 1). Only the N-*iso*propyl (compound 4) of the *p*-nitrophenyl N-alkylcarbamates resulted in a great selectivity difference for

the two cholinesterase types. Carbamates derived from heterocyclic enols were more active inhibitors of the butyryl-than of the acetylcholinesterase studied. A similar selectivity with certain of these heterocyclic derivatives has been previously reported (Gysin 1954, Pulver & Domenjoz 1951). The maximum selectivity occurred with 5,5-dimethyldihydroresorcinyl N,N-pentamethylene carbamate (compound 26), where more than 100,000 times the amount of carbamate was required for the same inhibition of the acetylcholinesterase compared with the butyrylcholinesterase. This is more selective than previous inhibitors used for differentiating these two cholinesterase types (Augustinsson 1957). 1-Naphthyl N-methylcarbamate (compound 17) displayed a selectivity for the acetylcholinesterase (Moorefield & Tefft 1958), a property not shared by its dimethylcarbamate analog (compound 18).

Toxicity of Certain N-alkyl and N,N-dialkylcarbamates.—The relationship of structure to insect toxicity for the carbamates shown in table 1 is similar to that reported in the extensive studies of Gysin (1952, 1954). Dialkylcarbamates derived from heterocyclic enols were more toxic to flies than those derived from aromatic enols and the N,N-dimethyl was the optimal substitution on the carbamoyl group. Remarkable selectivity in toxicity to house flies compared with mice occurred with some compounds which otherwise appeared quite similar (for example compounds 19 and 23).

The carbamates indicated in table 1 were generally more toxic to mice by the intraperitoneal route than to house flies following topical application. Some of the toxicity to mice may be due to hydrolysis products of the carbamates, particularly with the highly unstable *p*-nitrophenyl N-alkylcarbamates (compounds 1 to 4) since *p*-nitrophenol administered in the same way gave an LD_{50} of about 32 mg./kg.

Hydrolysis of p-*nitrophenyl N-alkylcarbamates.*—The stability of four *p*-nitrophenyl N-alkylcarbamates was compared with that of *p*-nitrophenyl acetate and ethyl carbonate and of *o*-nitrophenyl acetate. Spontaneous hydrolysis rates for the N-alkylcarbamate esters were 83 to 358 times greater than for the acetates or ethyl carbonate. Because of this rapid hydrolysis of the N-alkylcarbamate esters (half-lives of 5.9 to 8.9 min. at pH 7.80), all results for enzymatic studies are expressed as the ratio of the initial hydrolysis rate for the nitrophenyl ester in the presence and in the absence of the enzyme preparation (table 2). Enzyme preparations used were: rabbit plasma; swine plasma of high arylesterase activity (Augustinsson & Olsson 1959); fractions from human serum containing the albumin (Fraction V), cholinesterase activity (Fraction IV-6a) and arylesterase activity (Fraction IV-1); and chymotrypsin. The increase in hydrolysis rate of the nitrophenyl esters was always greater with the acetates and ethyl carbonate than with the N-alkylcarbamates. Of interest was the great specificity difference for hydrolysis of the *ortho-* versus *para*-nitrophenyl acetates by whole swine plasma compared with purified human serum cholinesterase. A marked difference also occurred in the activity of the human serum cholinesterase on the ethyl carbonate compared with the acetate of *p*-nitrophenol.

The hydrolysis of the *p*-nitrophenyl N-alkylcarbamates was unaffected by human serum cholinesterase or arylesterase preparations at 1.0 mg./ml. despite the high activity of these preparations, particularly the cholinesterase, on the acetates and ethyl carbonate. Only small differences occurred with varying alkyl group in the increase of hydrolysis rate for the N-alkylcarbamates in the presence of human serum albumin (Fraction V) and whole swine plasma. However, with rabbit plasma the increase in rate of hydrolysis of the *p*-nitrophenyl N-alkylcarbamates was greater with the longer alkyl substituents (*i.e.* N-methyl<N-ethyl<N-*n*-propyl or N-*iso*propyl). Electrophoresis of rabbit plasma (Casida & Augustinsson 1959) showed that the total activity in liberating *p*-nitrophenol from these N-alkylcarbamates migrated coincident with the albumin and with the activity in liberating 1-naphthol from 1-naphthyl N-methylcarbamate. The activity in liberating *p*-nitrophenol from the *p*-nitrophenyl N-alkylcarbamates was the same with respect to alkyl specificity for the whole rabbit plasma and the albumin component after electrophoretic separation.

Chymotrypsin did not affect the hydrolysis rates for the *p*-nitrophenyl N-alkylcarbamates at an enzyme level where a great increase in hydrolysis rate resulted with the nitrophenyl acetates and ethyl carbonate. The N-alkylcarbamates did not acylate the esteratic site of chymotrypsin as resulted with the acetates and ethyl carbonate (table 2), since only with the acetates and ethyl carbonate was a rapid initial liberation of about one mole of nitrophenate ion per mole of enzyme observed (Hartley & Kilby 1954, Gutfreund & Sturtevant 1956). It was also found that 1-naphthyl N-methylcarbamate was not hydrolyzed by chymotrypsin based on colorimetric analysis for 1-naphthol liberation. The effect of up to 5-hr. preincubation of chymotrypsin with certain carbamates at $2\times10^{-3}M$ on the subsequent activity in hydrolyzing nitrophenyl acetate was investigated. Neither the acetylation of the chymotrypsin nor the subsequent hydrolysis of the chymotrypsin acetate was affected by any of the following compounds: N,N-dimethyl- and N,N-diethylcarbamoyl chloride; N,N-dimethylcarbamoyl fluoride; Sevin, Pyramat, 3-methyl-5-pyrazolyl N,N-dimethylcarbamate; and 2,6-dimethyl-4-pyridyl N,N-dimethylcarbamate. When Pyrazoxon® (*O,O*-diethyl *O*-3-methyl-5-pyrazolyl phosphate) was used under identical reaction conditions, the rapid acetylation reaction on addition of nitrophenyl acetate was completely blocked. The N-alkyl and N,N-dialkylcarbamates failed to react with chymotrypsin in a manner analogous to that resulting with certain organophosphorus compounds (Hartley & Kilby 1952, Balls & Jansen 1952).

Purified preparations of lipase, papain, pepsin, trypsin, and egg albumin were also found to have no effect at 1.0 mg./ml. on the hydrolysis rate of *p*-nitrophenyl N-methylcarbamate in $0.05M$ pH 7.80 phosphate buffer.

DISCUSSION.—The toxicity of the carbamates to flies and mice did not correlate with their activity as inhibitors of human serum butyrylcholinesterase or *Torpedo* electric organ acetylcholinesterase. Such a correlation would not be expected since the toxicity to flies of substituted-heterocyclic N,N-dimethylcarbamates (Roan & Maeda 1954) and substituted-phenyl N-methylcarbamates (Kolbezen *et al.* 1954) is not closely related to the *in vitro* activity of these carbamates in inhibiting the brain

Table 2.—Hydrolysis of certain nitrophenyl N-alkylcarbamates, acetates and ethyl carbonate.

	o-NITROPHENYL ACETATE	p-NITROPHENYL ACETATE	p-NITROPHENYL ETHYL CARBONATE	1[a]	2[a]	3[a]	4[a]
SPONTANEOUS HYDROLYSIS							
$K_{hyd.}$ (min.$^{-1}$), pH 7.80 phosphate	6.7×10^{-4}	9.4×10^{-4}	3.3×10^{-4}	7.8×10^{-2}	10.0×10^{-2}	9.8×10^{-2}	11.8×10^{-2}
ENZYMATIC HYDROLYSIS							
Ratio: $enz.k_{hyd}/spont.K_{hyd.}$							
Rabbit plasma—10 μl./ml.	189	141	114	2.47	3.30	5.58	5.57
Swine plasma—10 μl./ml.	21	684	445	1.60	2.24	2.13	1.61
Human serum fractions—1.0 mg./ml.							
Albumin (V)	39	31	62	3.69	3.82	3.84	3.16
Arylesterase (IV-1)	13	17	6	1.00	1.00	1.01	0.98
Cholinesterase (IV-6a)	4,950	107	1,750	0.98	1.03	1.02	0.98
Chymotrypsin—1.0 mg./ml.	289	300	206	0.98	1.00	0.99	0.99
Acylation of Chymotrypsin	+	+	+	—	—	—	—

[a] Numbers refer to compound designations for p-nitrophenyl N-alkylcarbamates shown in table 1.

cholinesterase from the same species of flies. However a direct relationship has been reported between contact toxicity to thrips of substituted-phenyl N-methylcarbamates and their activity as *in vitro* inhibitors of house fly brain cholinesterase (Kolbezen *et al.* 1954).

All the toxic N,N-dimethylcarbamates fell within the range for second order hydrolysis constant of 8×10^{1} to 4×10^{-3} K_{OH} (1. mole^{-1} min^{-1}). Within this stability range no consistent relationship is evident between toxicity and *in vitro* anticholinesterase activity as determined with the serum butyrylcholinesterase or electric organ acetylcholinesterase. For a series of substituted-phenyl N-methylcarbamates, Kolbezen *et al.* (1954) found an inverse relationship between hydrolysis rate and both anticholinesterase activity and toxicity. The most toxic compounds in the present study were more stable than any of the carbamates considered by Kolbezen. Apparently the requirement for a high degree of stability to hydrolysis is met by the N,N-dimethylcarbamates shown in table 1 and other factors are more dominant in determining the toxicity of individual compounds.

The N-alkyl and N,N-dialkylcarbamates are considered to be active as cholinesterase inhibitors because of an ability to compete effectively with choline esters for the active site of cholinesterase. The rate at which the equilibrium between inhibitor, cholinesterase, and choline ester is reached varies greatly with different carbamates, but they are generallly thought to be completely reversible in this competition. However, certain workers (Myers 1954, 1956, Myers & Kemp 1954, Bergmann 1958) consider that the carbamates form carbamoyl derivatives of cholinesterase at its esteratic site in a manner similar to that in which dialkyl phosphate esters phosphorylate the active center. Myers *et al.* (1957) on reconsidering the available evidence changed their view to favor competition rather than carbamoylation as the mechanism of inhibition. From the present study it can be noted that the N-alkyl and N,N-dialkylcarbamates do not acylate chymotrypsin as do acetate, ethyl carbonate and dialkyl phosphate esters. Further, the butyrylcholinesterase of human serum does not hydrolyze p-nitrophenyl N-alkylcarbamates at a detectable rate, even though these esters are very unstable and are not highly inhibitory to the cholinesterase at the carbamate concentrations used in studying the hydrolysis. The relationship between the stability, anticholinesterase activity and insect toxicity is greatly different for the substituted-phenyl N-methylcarbamates and dialkyl phosphates (Metcalf 1955). If the carbamates do indeed form a carbamoylated derivative of cholinesterase analogous to the phosphorylation by phosphate esters, then this reaction must occur very slowly compared with the rate of formation of the carbamate-cholinesterase complex, and it would appear to be of secondary importance in the inhibition process with most carbamates.

Several different enzyme systems appear to be involved in the detoxification of N-alkyl and N,N-dialkylcarbamates. Although cholinesterase will apparently hydrolyze certain carbamates (Goldstein & Hamlisch 1952, Myers 1952, 1956), the turnover number is so low that this enzyme cannot be considered of importance as a major detoxification mechanism. Plasma albumin has been implicated as a factor of possible importance in detoxification of 1-naphthyl N-methylcarbamate (Casida & Augustinsson 1959) and p-nitrophenyl N-alkylcarbamates. However, plasma albumin does not readily hydrolyze the more stable, N,N-dialkylcarbamates. The least stable of the N,N-dimethylcarbamates included in this study, the fluoro derivative, is hydrolyzed by enzyme(s) in rabbit plasma which may also be involved in the hydrolysis of di*iso*propyl phosphoryl fluoride. In rabbit liver and kidney different enzymes are primarily involved in the hydrolysis of N,N-dimethylcarbamoyl fluoride and di*iso*propyl phosphoryl fluoride (Augustinsson & Casida 1959). Yet other systems must also be involved in the detoxification of the even more stable N,N-dimethylcarbamates derived from aromatic and heterocyclic enols, since poisoned animals recover from symptoms produced by sublethal doses of these compounds at a greater rate than would result only from excretion of the intact molecule. The biochemical nature of these alternate detoxification systems is not known.

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A Preliminary Report on Mutations in the House Fly[1]

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ABSTRACT

The origin of several mutant types in the house fly, *Musca domestica* L., is presented. Eleven mutants studied, and which behave as simple recessives, are described. Several aberrations that have not been studied thoroughly are also described. Many of these are no doubt valid mutants, but until further tests have been completed mutant names will not be assigned to them.

This paper is one of a series on the genetics and ethology of DDT-resistance in *Drosophila* and house flies, *Musca domestica* L., from the Department of Entomology of the University of Kansas.

Recently, interest in the study of the genetics of the house fly has increased owing to the discovery of insecticide resistance in this organism. Milani (1956) reported a number of morphological mutants. In our work a search of laboratory and field strains has been carried out in order to find mutants useful for marker stocks. This report gives preliminary information on several mutations and aberrant types which have been found in our survey. While the number of aberrant types found is very large, the number of mutants which are of high penetrance and useful as genetic markers is few. Certain anomalous types occur with great frequency and seem to be of world-wide occurrence; Milani in Italy, Tsukamoto *et al.* (1957) in Japan, and we have found many of the same aberrant characters. We have not compared our mutants by breeding tests with those of other investigators.

METHODS AND MATERIALS.—The single-pair culture techniques used in this work are described by Sullivan (1960). All flies were reared by single-pair culture techniques involving brother-sister mating for an F_2 generation. The laboratory strains that were examined for mutants include those listed by Sokal & Hunter (1955), an the D^{+++} strain and IS-1 strain obtained from W. N. Bruce, of the Illinois Natural History Survey, Champaign. Several field collections of mated flies were made at various localities in Kansas. Those studied most extensively include two field collections from the city dump in Ottawa, Kansas. The following discussion is based primarily on one of these Ottawa collections.

RESULTS.—Inbreeding of field-collected flies and laboratory strains has yielded a large number of mutant types. From 19 original parent females collected at Ottawa 5 F_2 cultures were set up per female and 83 F_2 cultures were obtained. Of these 43 showed some aberrant flies. All aberrant individuals were mated to brothers or sisters showing the same abnormality, or were crossed to wild type flies and inbred in attempts to establish mutant strains. In many cases the aberrations were not recovered again; in other cases they were recovered, but the frequency of abnormal types was much lower than expected for a recessive mutant. Penetrance was frequently low and the degree of expression of mutant characters was found highly variable. As an example of this variable expression the character *break* (*bk*), which causes a break in the posterior cross vein, has been inbred by brother-sister matings for more than 20 generations, and at this time the expression varies from a complete absence of this vein to only a short interruption of the vein. Most of the wing-vein breaks described in the list of mutants and in the list of aberrations behave in this manner. Many of the mutants, including broken veins, have been found useful in testcrosses. The above character, *bk*, has been found at numerous times in both field and laboratory cultures of different origins. Frequently the same aberrations have been found in different strains.

Table 1.—Comparison of the number of mutant types found by Milani (1956) on collections in Italy and in F_2 progenies from collections made at the Ottawa, Kansas, city dump.

No. of "Mutations" Recovered from Each Female	No. and Origin of Females		
	Tripoli, Italy	Latina, Italy	Ottawa, Kansas
0	2	1	2
1	2	4	6
2	1	3	3
3		1	1
4		1	4
5		2	1
6		1	2

The number of aberrant types in the Ottawa field collection has been mentioned. Further note may be made of the distribution of these aberrant types with respect to their origin. Table 1 indicates a comparison of the number of mutant types found in the F_2 offspring descended from single females. The results of Milani on two Italian collections are presented to give an indication of their similar high variability. Different types of aberrations found in the Ottawa group numbered 18. In the descendants of 10 of the females, flies with *pigmented sternites* (*ps*) were found, indicating a very high frequency of this recessive character in this locality. Most of the aberrants did not appear in the number expected of at recessive mutant, but the high familial incidence indicates that they were heritable, rather than developmental, anomalies. These characters are included in the list of aberrations. Only those that yielded expected recessive ratios are included in the list of mutants.

Many of the aberrant types described herein have been discarded. All mutants were kept for several generations of inbreeding in order to establish that they were heritable. If at this time the penetrance had not increased and outcross tests did not yield aberrants, the strains were discarded in many cases. Whenever aberrations are listed the familial incidence of the characters was sufficient to convince us of their heritable nature. Only those which in testcrosses and outcrosses were shown to behave as recessive mutants are so marked and given symbols. Linkage studies are currently being performed.

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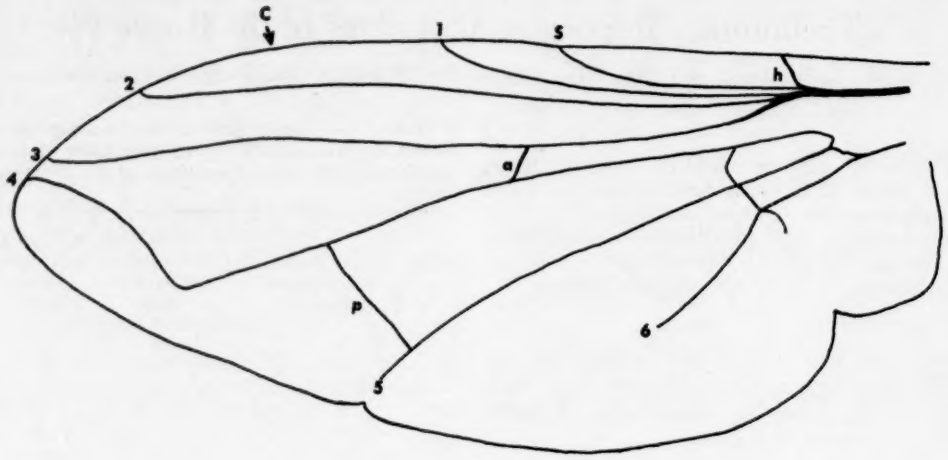

FIG. 1

In the following lists the nomenclature of the wing veins is taken from West (1951 p. 31); figure 1 illustrates the normal wing.

TESTED MUTANTS

break (*bk*): the posterior cross vein is interrupted or entirely absent in this strain. Pure stocks have been derived from several sources and are currently being tested. Resembles *abruptex* of Milani. Recessive.

clump (*cp*): the fourth longitudinal vein is heavily thickened in the region between the two sharp curves of this vein. This thickening is usually very heavy, occasionally only a small hook of extra vein is found in this region. Recessive.

fin (*fn*): on the fourth longitudinal vein between the two sharp curves of this vein there is formed a small triangular fin which projects from the ventral surface of the wing. The base of this fin is approximately one-quarter of the length of vein L_4 beyond the posterior cross vein. Recessive.

giant (*gi*): the larvae and pupae of flies are about twice normal size. No adult flies have been obtained from this strain. Tests have involved normal brothers and sisters heterozygous for the character. Pupae are usually abnormal, elongated, of approximately the same shape as the full grown larvae, sometimes pupal cases are normal in shape but large.

interrupted (*it*): vein L_4 is broken in the region where it curves upward toward the costal margin of the wing, usually not extending to the wing margin. May occur as two breaks. Recessive.

ocelliless (*oc*): the entire ocellar triangle is absent, including the ocelli and ocellar bristles in most cases. Occasionally the ocellar triangle is present, but displaced from its medial location lying close to one eye, with fewer ocellar bristles and ocelli. Recessive.

pigmented sternites (*ps*): the sternites of the abdomen are black, especially in the male. In young flies the sternites and ventral border of the tergites are heavily outlined in black. With increasing age the amount of black pigment increases until the entire sternite is black. Recessive.

shaven (*sh*): adult flies have the bristles of the head, thorax, and abdomen reduced; a rough stubble appearance is achieved. Adult flies showing this character frequently have curled wings and abnormalities of the legs. Survival of adults to reproduction has not been achieved. Frequently emergence from the pupal case is not successful. Recessive.

subcostal break (*sb*): the subcostal vein is interrupted or very thin for varying portions of its length. Selection has yielded a pure strain of this mutant which is being used in testcrosses. Found in field and laboratory strains. Phenotypically resembles the mutant *b* of Lichtwardt *et al.* (1955).

swollen (*sw*): a leg abnormality causing a shortening and/or swelling of the femora and tibiae, frequently not affecting all the legs of an individual. Emergence from the pupa is frequently not successful. Adults are fertile. Recessive, highly variable in expression.

unexpanded (*ux*): the wings do not expand when the flies emerge. This anomaly is frequently found as an environmental effect, however strains have been selected in which the character is expressed in all flies. Testcrosses indicate a recessive character of poor penetrance. Discarded owing to variability in testcrosses.

ABERRATIONS CURRENTLY UNDER STUDY

aristapedia—the pedicel of the antenna develops into a roughly triangular shape; the arista is reduced, absent, swollen or tarsuslike. Poor penetrance and usually unilateral in expression.

anterior cross veinless—the anterior cross vein is either absent or broken in flies of this strain.

bald—the inner or outer set of vertical bristles is absent, found frequently in different strains.

bite—the wings along the rear margin show small indentations or scallops. Highly variable in expression, probably recessive.

bristle—at the juncture of the humeral and subcostal veins a bristle arises on the dorsal surface of the wing. This character is of low penetrance and frequently affects only one wing.

broad—the third longitudinal vein is thickened for a portion of its length. Selection has not yielded a pure strain.

broad anterior cross vein—the anterior cross vein is thickened for its entire length. Resembles *brcr* of Milani. Pure stock has been developed but not tested.

broad vein—the posterior cross vein is thickened and of irregular outline. Currently under selection for a pure strain.

bubble—wings of the adult flies contain bubbles of fluid, phenotype resembles the *balloon* mutant of Milani. A strain has been selected in which this character is expressed in more than 90% of the flies.

corrugated—the wing is folded from the base to the tip giving a slightly wrinkled wing that is otherwise normal. Recessive in preliminary tests.

costal break—the costal vein is interrupted at one or more places. Wing shape is frequently affected, showing a slight folding. Point of breakage usually occurs between the subcostal and first longitudinal veins.

curled—the wing tips are curled downward, poor penetrance in F_2 tests.

curly—the wing tips are curled upward, selection for a pure strain has not been successful.

ebony—the characteristic longitudinal lines on the dorsal surface of the thorax are absent, leaving a solid black surface. F_2 results indicated recessiveness, but the strain was lost before tests could be completed.

entire—the fifth longitudinal vein is extended to meet the margin of the wing. The margin at the point of juncture is smooth, lacking the normal notch.

extra veins—extra small veins arising from the posterior cross vein, resembles the *plexus* mutant of Milani.

folded—the wings are folded under so that the tip almost touches the thorax. Frequently coincident with wing folding the hind legs are gnarled or swollen. High incidence in certain families but not recovered in F_2 of outcrosses.

interrupted L_4—vein L_4 is interrupted or thin for a greater or lesser part of its length before it intersects with the anterior cross vein. Resembles *gap* of Milani.

interrupted L_5—the fifth longitudinal vein is broken for a portion of its length prior to the point where it meets the posterior cross vein.

plane—the wings are held out from the body of the resting fly. Resembles *divergent* of Milani.

reduced eye—one or both eyes is either divided longitudinally or reduced, or altogether absent. A strain has been isolated from a single aberrant fly and after eight generations of selection yields about 20% aberrant flies.

tipless L_3—the third longitudinal vein is interrupted close to the margin of the wing.

tipless L_2—the second longitudinal vein is interrupted at the margin of the wing, usually a loss of less than 5% of the vein. Frequently expressed in only one wing. A pure strain has been derived, but behavior in crosses has not been tested.

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Estimating Immature Populations of Rice Weevils in Wheat by Using Subsamples[1]

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ABSTRACT

Wheat was infested at three levels with rice weevil (*Sitophilus oryza* (L.)), differing in age. Each level of infestation was replicated three times. Each replicate was subdivided six times and one division sampled four times when the organism was in a particular immature form. Eventually all six divisions were sampled.

Immature infestation was determined by counting adult emergence from samples. Various factors affecting emergence were studied by a mixed classification (factorial, nested) analysis of variance. It showed: (1) age of cultures used had no effect on total emergence; (2) level of infestation accounted for more than 63% of total variation in the experiment; (3) that greater efficiency could be obtained by using a single source of infested wheat; and (4) that consistency can be improved by taking samples for control and treatment from a population that contains a relatively high percentage of one stage. A table useful in estimating the number of samples necessary to obtain reliable estimates of immature infestation is given.

Nearly all fumigant-evaluation tests on internal grain-infesting insects use emerged adult insects as the criterion to measure mortality. The importance of internal infestation in grain is well known, but the effects of fumigant formulations on the internal forms have been little tested. A probable reason for the scarcity of information on the immatures is that determination of their mortality is difficult. This problem could be reduced by having an accurate estimation of such infestation.

Adkisson (1957) determined per cent immature mortality by subtracting treatment emergence from control emergence and dividing the remainder by control emergence. He used four 250-kernel replicates from a "thoroughly" mixed infested wheat. Krohne & Lindgren (1958) used 100-gram aliquots of infested wheat for fumigation tests with similar aliquots for controls as indices of infestation. They did not, however, mention the number of samples used to estimate the control infestation. Pedersen (1959) used two cages containing 10 grams of infested wheat for each of three replicates. Total emergence from six control samples was compared with total emergence from six treated samples at each dosage. Per cent mortality was determined in a manner similar to Adkisson's.

How many samples are necessary to estimate sample infestation with specified confidence? Tests were conducted to: (1) determine the number of subsamples necessary to estimate immature rice weevil infestation; (2)

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estimate the effects of three levels of infestation; (3) estimate the effects of two adjacent ages of parent stock; (4) estimate replicate variation; and (5) estimate subsample variation.

MATERIALS AND METHODS.—Insect-free 1958 Ponca wheat, held in cold storage (ca. 40° F.) until used, was conditioned to 80° F. and 13.5% moisture. Five-hundred-gram lots of wheat were placed in each of 18 one-gallon, narrow-mouthed jars. Each jar was fitted with a 60-mesh wire-screen lid, lined with filter paper to exclude other insects.

The 18 one-gallon jars were separated into three groups and infested with adult rice weevil (*Sitophilus oryza* (L.)) at the following levels: Group I—300 insects each; Group II—500 insects each; and Group III—700 insects each. The weevils were obtained from standard laboratory cultures, *i.e.*, reared in wheat at 80° F. and 70% relative humidity from eggs deposited during a 3-day period. The insects were obtained from cultures, designated A and B, started 51 and 48 days, respectively, prior to use. Three lots of wheat from each group were infested with insects from culture A; and three lots with insects from culture B. This method provided three replicates at each level of infestation for each age of culture.

Jars of wheat containing the various levels of infestation were placed in a rearing room at 80° F. and 70% R. H. for 3 days of oviposition, after which all parent adults were screened from the wheat. Wheat from each gallon jar (lot) was divided into six 80-gram divisions and placed in 1-quart, wide-mouthed jars. Excess wheat was discarded. Each jar was covered with a screened lid lined with filter paper. Divisions in quart jars were stored in the rearing room and retained to develop immature insects and for subsampling. Samples of the immature forms were taken on the following schedule:

Immature Form	*Sampling Time (Days After Start of Oviposition)*
Eggs (E)	4
1st instar larvae (I)	8
2nd instar larvae (II)	12
3rd instar larvae (III)	16
4th instar larvae (IV)	21
Pupae (P)	25

At each of the sampling times, one 80-gram division was taken from each replicate for each age (cultures A and B), at each level of infestation. Four samples of approximately 10 grams each were taken from each 80-gram division by means of a small glass vial. Divisions were mixed thoroughly prior to sampling. Each sample was placed in a plastic box ($1\frac{7}{8}'' \times 1\frac{7}{8}'' \times \frac{3}{4}''$), appropriately labeled, and returned to the rearing room for development of the insects.

Immature infestation was determined by counting adult insects emerging from each sample. The first count was made 31 days after the start of oviposition, with subsequent counts 7, 14, and 21 days later. Total emergence was determined by summing adult emergence from the four counts.

RESULTS.—Total emergence from samples for each age of parent and level of infestation is given in table 1.

A mixed classification (factorial, nested) analysis of variance was used to separate the effects of age of adult and level of infestation and to estimate the variance components due to various samplings. An outline of the component analysis with degrees of freedom is given in table 2. This table shows that ages and levels of infestation are factorial with replications, divisions, and samples nested within ages and levels.

Table 1.—Total emergence of rice weevil adults from samples taken from different ages and levels of infestation (number of parents per 500 grams of wheat) when immatures were in different stages.

Age of Parent Adults	Level of Infestation	Stage of Immature					
		Egg	I	II	III	IV	P
A	300	266	281	283	276	248	281
B	300	283	287	287	292	320	349
A	500	356	366	379	387	389	416
B	500	433	384	453	375	400	445
A	700	505	516	501	538	565	539
B	700	590	574	557	570	642	629
Total		2433	2408	2460	2438	2564	2659

Table 2.—Components of mean square of the constructed experiment.

Source of Variation	D/F	Expectation of Mean Square
Age (A)	1	$1\,\sigma^2_{s:drla} + 4\,\sigma^2_{d:rla} + 24\,\sigma^2_{r:la} + 216\,\sigma^2_{a}$
Level of infestation (L)	2	$1\,\sigma^2_{s:drla} + 4\,\sigma^2_{d:rla} + 24\,\sigma^2_{r:la} + 144\,\sigma^2_{l}$
A×L	2	$1\,\sigma^2_{s:drla} + 4\,\sigma^2_{d:rla} + 24\,\sigma^2_{r:la} + 72\,\sigma^2_{al}$
Replications (R): LA	12	$1\,\sigma^2_{s:drla} + 4\,\sigma^2_{d:rla} + 24\,\sigma^2_{r:la}$
Divisions (D): RLA	90	$1\,\sigma^2_{s:drla} + 4\,\sigma^2_{d:rla}$
Samples (S): DRLA	324	$1\,\sigma^2_{s:drla}$

Table 3.—Analysis of variance (mixed model) with F-tests and significance.

Source of Variation	D/F	Mean Square	F	Statistical Significance
Age (A)	1	1,401.12	4.6216	ns
Level (L)	2	18,825.60	62.0958	**
A×L	2	100.49	.3314	ns
Replications (R):LA	12	303.17	6.2381	**
Divisions (D):RLA	90	48.60	1.3019	*
Samples (S):DRLA	324	37.33		

* $P < .05$.
** $P < .01$.

The complete analysis of variance is shown in table 3. Levels of infestation account for a major part of total variation (63.4%). The F-test suggests differences in total emergence from grain infested with three levels of adults. Average emergences from 10-gram samples at each level of infestation were: 300 parent weevils, 72; 500 parent weevils, 100; and 700 parent weevils, 141.

Differences in emergence from replications within level and age are shown by the F-test. Such differences may be the result of unequal male-female ratios in lots during the oviposition period. Significance is shown for D:RLA at $P \leq .05$. This component measures the effects of sam-

Table 4.—Ranked mean emergence from divisions sampled at various stages of immature development.

STAGE OF DEVELOPMENT	I	E	III	II	IV	P
Ranked mean emergence[a]	401	406	406	410	427	443

[a] Means underlined are not significantly different, mean separation was achieved by the LSD* = 7.

pling one of six divisions taken from each replication when the immature insects were in various stages of development. Table 4 illustrates where differences occur between divisions. Clear significance in total emergence is shown between divisions sampled at the fourth instar and pupal stages. Fourth instar and pupal emergence were also different from emergence at the earlier stages. The relationship between emergence and stage of insect at sampling time is shown in figure 1.

Numbers of samples necessary to obtain an estimate of internal infestation were computed by use of the variance component $\sigma^2_{s:drla}$. Following the method of confidence intervals, *i.e.*, $ts_{\bar{x}} = d$, where $d_i = \bar{x}_i - \mu$. The information in table 5 was compiled by using selected sample sizes and probabilities of a Type I error (α). The estimated absolute difference between the sample mean and the population mean is given for indicated sample sizes and levels of probability.

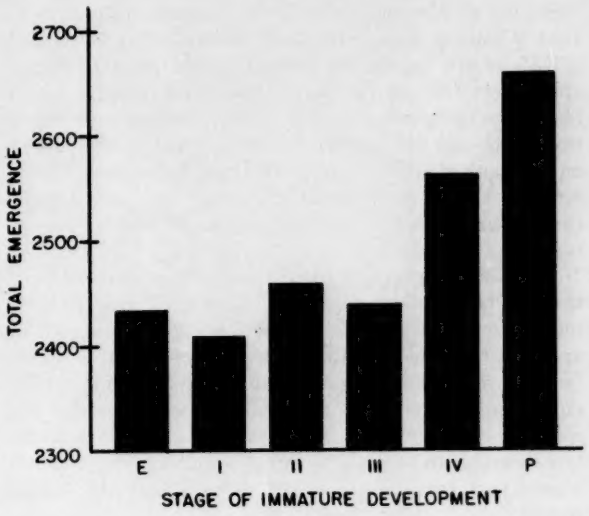

FIG. 1.—Total emergence of rice weevil (*Sitophilus oryza* (L.)) adults from divisions sampled at various stages of immature development.

Table 5.—Expected sample deviations from population infestation mean at various probabilities when different numbers of samples are observed.

SAMPLES	PROBABILITY OF TYPE I ERROR			
	.10	.05	.02	.01
3	13	19	30	43
4	9	12	17	21
5	7	8	11	14
6	6	8	10	12
7	5	6	8	9
8	5	6	7	9
9	4	5	6	7
10	4	5	6	7
12	4	5	6	6
14	4	4	5	6
16	3	4	5	5
18	3	4	4	5
20	3	3	4	5
∞	1	1	1	1

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Control of the Woolly Apple Aphid on Roots of Apple Nursery Stock[1]

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ABSTRACT

Suspensions of BHC, lindane, and aldrin applied at 20 pounds and of dieldrin at 40 pounds per acre to the soil surface in a band about the tree rows a few days after planting gave complete protection against woolly apple aphids (*Eriosoma lanigerum* (Hausm.)) on the roots of apple nursery stock without injuring the trees. Unfortunately, such heavy dosages are impractical and lighter ones failed to give complete control. Heavy dosages of demeton and chlordane were also ineffective. Dipping the roots of apple seedlings into pastes made up of 1 to 2 teaspoonfuls of BHC (10% gamma isomer), 50% dieldrin, an aldrin wettable powder containing 25% of the active ingredient with 1½ pounds of Attaclay or charcoal in 1 quart of water reduced the infestation and did not injure the trees but was cumbersome to carry out. In trunk-spray experiments moderate practical dosages of BHC, Sevin® (1-naphthyl methylcarbamate), demeton, and Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate), (about 6 to 10 ounces per acre) near the ground and to a narrow band of soil about the tree rows gave an encouraging degree of control and caused no injury. The minimum effective dosages remain to be determined. Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate), schradan, and Nemagon (1,2-Dibromo-3-chloropropane) were less effective.

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Studies at Kearneysville, West Virginia, from 1949 to 1951 (Gould & Hamstead 1951) showed that BHC and chlordane are capable of protecting the roots of young apple trees from infestation by the woolly apple aphid (*Eriosoma lanigerum* (Hausm.)). This finding encouraged work with soil insecticides for the control of that insect on the roots of apple nursery seedlings. Infestation affects 50% of the trees in some plantings and occasionally causes nurserymen to lose as many as 40% of 2-year-old trees.

Apple-seedling grafts usually remain in nursery beds through two growing seasons and are then dug for shipment as orders are received. Row spacing and tree growth are such that conventional sprayers are not suitable for treating such plantings. It is necessary to use special equipment or to adapt conventional machines for the purpose, especially during the second season. It is therefore desirable to control the aphids so well during the first season that treatment will not be necessary the second season.

Aphidicides applied to the trees in normal spraying operations have little effect on subsoil populations in apple nurseries. Siegler (1953) found that distribution of BHC in the open furrow just before planting killed all the seedlings. A study was therefore made of various insecticide formulations and methods of applying them.

Test plots were set up in a Latin-square design, with eight replications, in commercial nursery beds at Waynesboro, Virginia, and Aspers, Pennsylvania. Each replicate usually consisted of 50 trees. Tree mortality, regardless of treatment, was sometimes high, thus reducing the number of trees available for examination at the end of the second season.

Table 1.—Control of the root-infesting form of the woolly apple aphid on apple nursery stock with soil-surface treatments, Waynesboro, Virginia, 1953–54.

Insecticide[a]	Dosage in 2 Gal. per 25 Feet of Row	Root Systems	
		No. Examined	Infested (%)
Demeton 48%, EC	14.8 ml.	74	63.2
	29.6	88	46.6
	59.2	116	63.0
Demeton	10[b]	148	4.0
	29.6[b]	160	5.0
Chlordane 40%, WP	17.2 grams	81	38.3
	35.8	101	31.7
	71.6	80	13.8
BHC (10% g.i.) WP	71.6	87	1.2
	143.2	85	0
	286.4	84	0
Dieldrin 25%, WP	28.4	82	13.5
	56.8	77	10.4
	113.6	72	0
Lindane 25%, WP	19.3	104	2.0
	57.3	122	0
Aldrin 25%, WP	19.3	109	2.0
	57.3	175	0
Untreated		575	41.0

[a] One application (1953) unless otherwise indicated.
[b] One application at beginning of each growing season.

Table 2.—Control of the woolly apple aphid on apple nursery stock with root-dip treatments, Waynesboro, Virginia, 1954–55.

Insecticide	Teaspoonfuls in 1 Quart of Water with 1½ Pounds of Attaclay	Root Systems	
		No. Examined	Infested (%)
BHC (10% g.i.) WP	1	75	20.0
	2	110	9.1
Dieldrin 50%, WP	1	271	48.5
	2	144	16.6
Aldrin 25%, WP	1	184	17.3
Untreated	—	128	23.4

Soil-Surface Treatments.—In April 1953, suspensions of insecticides were applied with a sprinkling can to the soil in a band 1 foot wide about the tree row after the trees had been planted. Two gallons were applied to each 25 feet of row of 50 trees. Treatments were completed within a few days after planting and before the soil surface became hardened.

The treatments used and results taken in October 1954 at Waynesboro are given in table 1. At Aspers, aphids did not develop in sufficient numbers to give significant results. At Waynesboro, 143.2 grams of BHC (10% gamma isomer) and 57.3 grams each of 25% lindane and 25% aldrin wettable powders per 25 feet of row (20 pounds of toxicant per acre) and 113.6 grams of 25% dieldrin wettable powder per 25 feet of row (40 pounds of toxicant per acre) gave complete control without injuring the trees. Unfortunately, such high dosages are expensive and might have injurious effects on subsequent use of the nursery beds, especially if long-continued. It was encouraging that lower dosages of these materials also reduced infestation markedly, although they were not completely effective.

Root Dips.—The first attempts to obtain control with root dips were unsuccessful. The suspensions either injured the roots or were ineffective. Attaclay was then used as a carrier and safener. The insecticides were mixed with water and Attaclay was added to make a thick creamy paste into which the roots were dipped just before planting. In preliminary tests 1½ pounds of Attaclay per quart of water was found to give the most satisfactory consistency. The results with these paste dips are given in table 2.

The most effective dip, which contained 2 teaspoonfuls of BHC (10% gamma isomer), caused a substantial reduction in infestation but not enough to be satisfactory. There was no indication that any of the dips injured the trees.

In a second series of tests activated charcoal was used as the carrier-safener. The aphid infestation was too low to yield significant results in control, but no evidence of injury from the treatments was found.

There are serious disadvantages to the root-dip treatment. Nurserymen often prefer to use large seedling stock for planting, then budgraft the trees in August of the first growing season. Dips are cumbersome to use if the root

FIG. 1.—Effects of wind-sway on apple seedlings. The separation of the trunks from contact with surrounding soil facilitates the establishment of subsoil aphid colonies.

system of the seedling is 6 inches or more in diameter. In the process of handling and planting, a large amount of the mixture is smeared on planting equipment and on operators. Clearly a better method of treatment was indicated, and testing of root dips was discontinued.

TRUNK SPRAYS.—Infestations of the woolly apple aphid first appear on the tree trunks just below the soil level and down to the base of the first lateral roots one to several inches below the surface. Young trees are subjected to wind sway, which separates the trunks from contact with the soil, providing the aphids easy access to the root system (fig. 1). This condition suggested the use of a trunk spray applied before aphids have an opportunity to become deeply entrenched and to give protection at the site most vulnerable to infestation.

Tests of trunk sprays were begun at the Waynesboro nurseries in June 1955, several weeks after the trees had been planted. BHC, 40 grams of 10% gamma isomer per gallon of water, and dieldrin and demeton at comparable rates were applied with a 5-gallon knapsack sprayer with the nozzle adjusted to throw a narrow stream, directed on the tree trunk at ground level. These tests resulted in a sprayed band 2 to 3 inches wide on the soil surface. Five gallons of spray was sufficient to cover about 300 yards of row, or 1,800 trees. Only one application was made.

Results were very promising. Examination of approximately 100 trees per treatment showed the following numbers infested: BHC 0, dieldrin 2, demeton 1, and untreated 24.

Trunk-spray experiments were continued in nurseries at Aspers and Waynesboro in 1958. There were seven treatments and a check in an 8×8 Latin-square plot arrangement. Applications were made on June 30 at Aspers and on July 2 at Waynesboro with a knapsack sprayer as in 1955, using 2 gallons of spray, or 1 quart per replicate of 50 trees.

The effectiveness of the treatments was determined the last week in October. No significant results were obtained from the tests at Waynesboro owing to natural mortality of trees, loss by removal in nursery operations, and lack of infestation. At Aspers, examination of the roots of 1,881 trees revealed that the aphid infestation was generally light and irregular and ranged from very light to moderate. Percentages of trees infested were as follows:

	Fluid ounces in 2 gallons	*Per cent Infestation*
BHC, 11% gamma EC	0.75	3.5
Sevin® (1-naphthyl methylcarbamate) 13% EC	1	4
Guthion® (*O,O*-dimethyl *S*-(4-oxo-3 *H*-1, 2,3-benzotriazine-3-methyl) phosphorodithioate) 17.3% EC	0.5	5
Demeton, 26.2% EC	0.5	5
Trithion® (*S*-(*p*-chlorophenylthio) methyl *O,O*-diethyl phosphorodithioate) 50% EC	0.5	9
Nemagon (1,2-Dibromo-3-chloropropane) 50% EC	0.25	10.6
Schradan, 46% EC	0.5	11.4
Untreated		6

BHC, Sevin, Guthion, and demeton are considered promising for applications as trunk sprays and worth trial.

Trunk sprays have the following advantages over other methods thus far used: (1) A large number of trees can be treated with light low-pressure equipment, because the material is used at a low-gallonage rate; (2) the insecticide makes contact with the tree at the site of the infestation but does not come in contact with easily injured feeder roots; (3) a concentrated spray can be applied for longer residual protection with less danger of soil toxicity because only a small portion of the surface is treated. The amount of BHC used in the 1958 tests is equivalent to about 3½ pounds of the 11% material per acre; however, this dosage gave only moderate control. On the assumption that four times this concentration would be satis-

factory, the dosage would be equivalent to only 1½ pounds of the gamma isomer per acre.

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A Three-year Overwintering Study of the Spotted Alfalfa Aphid[1]

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ABSTRACT

During a 3-year study in the eastern third of Kansas, the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), survived one winter from the southern to the northern boundary of Kansas, and two winters as far north as Manhattan. Overwintering was restricted to south-facing slopes of roadside embankments when it occurred in the north and central portions but occurred in both fields and roadside embankments in the southern portion.

The spotted alfalfa aphid, *Therioaphis maculata* (Buckton), was first reported in extreme southwest Kansas in August 1954. By the fall of 1955 it had spread to all but five northeastern counties. Apterous adults and nymphs were found in the Manhattan area until January 14, and in southern counties as late as March of 1956 (Peters 1956). Certain fields in Montgomery County (southeast) showed heavy infestations and extreme damage by April 18. Only south slopes of hills and terraces showed infestation and damage. The alate form of aphids was absent, indicating overwintering. The purpose of this study was to determine the extent of overwintering and under what conditions it occurred.

MATERIALS AND METHODS.—Various types of alfalfa fields were selected for sample stations, such as those grazed by livestock, those exposed to and protected from wind, and tall and short growths going into winter. South-facing slopes of roadside embankments seeded to alfalfa to prevent soil erosion were also sampled. Selected sampling stations were located from Oklahoma to Nebraska in northern, central, and southern areas across the eastern third of Kansas. The northern area ranged from Nebraska to 15 miles north of Manhattan and was sampled at approximately 2-week intervals. The central area covered a 15-mile radius from Manhattan. This area was sampled each 7- to 10-day interval. The southern area extended from central Kansas to Oklahoma and was sampled at 3-week intervals.

Five samples comprising a total of 25 plants were collected at random from each station per sampling date. Plants were carefully cut with a sharpened garden trowel, taking a portion of soil and debris. The plants were carefully put into a paper bag, stapled, and placed as soon as possible in Berlese funnels for 3 hours. Previous trials with Berlese funnels proved that a lower aphid population could be detected by using this method than by sweeping or brushing. Aphids were collected below the funnels in a Mason jar half full of water. The contents were then poured into an enameled pan and the aphids counted.

Table 1.—Average monthly temperatures (° F.) of 3 areas in the eastern third of Kansas during spotted alfalfa aphid overwintering studies.

Month	1956–1957			1957–1958			1958–1959		
	North	Central	South	North	Central	South	North	Central	South
Dec.	35.1	34.8	37.8	38.1	37.4	41.0	30.5	29.7	32.7
Jan.	21.6	22.5	26.6	31.4	29.6	34.1	23.1	22.4	27.0
Feb.	35.8	35.1	40.1	27.2	25.0	30.0	29.5	30.1	34.1
Mar.	41.4	41.6	44.1	30.7	35.9	35.5	43.3	43.9	46.4
Apr.	51.0	51.8	53.8	51.0	52.7	53.9	52.9	53.2	55.6

Soil, plants, and weather conditions were noted at the time of sampling. Data from official weather stations in the three areas were averaged (table 1). Muddy samples resulting from snow or rain occasionally interfered with accuracy of counts. One additional collection was taken from each field following a zero count as assurance that aphids were no longer present. Samples were then collected from roadside embankments after aphids disappeared in all fields in an area. Aphids were continually present in fields of the southern area. Consequently, embankments did not require sampling. Temperatures of embankments were recorded at the time of sample collections, but these were inadequate to compare with field temperatures and obtain any correlation with aphid survival.

In the first year of study 38 fields and embankments were checked regularly for the presence of aphids. During that time more than 1,100 samples were collected, totaling more than 5,500 plants. A total of 25 fields and embankments was sampled regularly during the second season. Most of these stations were also used during the third year.

RESULTS.—*1956–1957.*—Aphids were present in fields of the northern area from the time initial samples were collected, December 28, 1956, until February 2, 1957 (table 2). Alfalfa fields in the central area contained aphids until March 29, 1957. The southern part contained aphids throughout the winter. The number was lowest (average of 5 per 25 plants) in mid-March but gradually increased to an average of 40 per 25 plants by late April.

Plants collected from roadside embankments in the

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Table 2.—Detection of overwintering spotted alfalfa aphids in three areas in the eastern third of Kansas.[a]

Month	1956–1957			1957–1958			1958–1959		
	North	Central	South	North	Central	South	North	Central	South
				Fields					
Dec.	+	+	−	+	+	+	+	+	+
Jan.	+	+	+	+	+	+	+	+	+
Feb.	+ (2/2/57)[b]	+	+	+ (2/21/58)	+ (2/24/58)	+	+ (2/7/59)	+ (2/14/59)	+
Mar.	0	+ (3/29/57)	+	0	0	+	0	0	+
Apr.	−	−	+	−	−	+	−	−	+
				Roadside Embankments[c]					
Mar.	+ (3/30/57)	+	−	0	+	−	+	+	−
Apr.	0	+	−	0	+	−	+	+	−

[a] + = Spotted alfalfa aphids present; − = unsampled; 0 = spotted alfalfa aphids not detected.
[b] Last date aphids detected.
[c] Sampled only after aphid populations disappeared from fields.

northern area, starting on March 9, yielded aphids until March 30, while samples from central roadside embankments averaged 179 per 25 plants throughout the remaining winter.

1957–58.—Alfalfa samples were first collected from fields in mid-December and continued through mid-March in the north and central areas. Aphids were detected until February 21 in the north and until February 24 in the central area. Populations were lowest in late March (averages of 7 per 25 plants) but present throughout the winter in fields of the southern area. Aphids were not detected at any time on northern embankments. They were present (average of 19 per 25 plants) on central embankments throughout the sampling period.

Frequent rains during the late summer and fall of 1957 apparently created favorable conditions for a fungus that infected the aphids. Fungi specimens submitted for identification could be determined only to Order Entomophthorales because of bacterial contamination. The fungus decreased aphid populations, going into the winter season. Hence, rather than selecting from previously established fields, it was necessary to sample any available infested field. Sampling stations in the Manhattan area were especially difficult to locate.

1958–59.—Plant collection started during the first week of December in all three areas. Initial populations during the fall of 1958 were higher than the previous two seasons (table 3). Twenty-five fields and roadside embankments were sampled.

Aphids were detected in northern fields until February 7, and in central fields until February 14, 1959. Samples from southern fields showed aphids present throughout the winter. Populations were lowest (average of 5.7 per 25 plants) during March but increased to an average of 22.4 per 25 plants by the last sample date on April 5. Both northern and central embankments, sampled from late March until late April, contained populations throughout the sampling period.

DISCUSSION.—Studies conducted during December through April of the 3 years have indicated the aphid successfully overwintered in the eastern third of Kansas. In 1956–57 and 1957–58 aphids overwintered as far north as Manhattan on south slopes of roadside embankments. In the southern area aphids overwintered both in fields and embankments, although populations were always low in fields. In 1958–59 aphids overwintered from Oklahoma to Nebraska. They survived winters in the north and central areas on south slopes of roadside embankments, while surviving both in fields and embankments in the southern area.

Average monthly temperatures were lowest during January of the first (1956–57) and last (1958–59) seasons of study (table 1), while February was the coldest month of the 1957–58 winter season. Populations, both apterous and alate forms, in fields decreased rapidly from December to February, but did not disappear until February or later, as shown in table 2. Alate aphids could be found until about the first week in February of each year. Later

Table 3.—Population decline of spotted alfalfa aphids as shown by mean monthly counts from initial to final sampling in the most heavily infested field in each of three areas in the eastern third of Kansas.

Month	Total Number of Aphids[a]				
	North		Central		South
	Field	Embankment	Field	Embankment	Field
		1956–1957			
Dec.	687	—[b]	1,555	—	3,926
Jan.	487	—	1,344	—	22
Feb.	52	—	31	—	27
Mar.	0	7	1	806	61
Apr.	—	0	0	596	112
		1957–1958			
Dec.	57	—	12	—	108
Jan.	9	—	9	—	172
Feb.	1	0	4	—	62
Mar.	0	0	0	22	35
Apr.	—	—	—	80	1
		1958–1959			
Dec.	4,600	—	5,392	—	26,270
Jan.	80	—	191	—	3,168
Feb.	4	—	21	—	24
Mar.	0	62	0	42	12
Apr.	—	60	—	144	83

[a] Zero does not necessarily indicate the absence of aphids in all fields in the area.
[b] — = Unsampled.

all forms were apterous with all instars represented. During late March or early April the alate form would reappear, gradually increasing in numbers as the season progressed.

Aphid survival of winter conditions was not affected by the height of alfalfa plants, or by fields exposed to, or protected from, windbreaks such as trees and haystacks. Data from alfalfa fields grazed by livestock during the fall were insufficient to determine the effect on populations. On several occasions aphids temporarily increased in number when snow covered alfalfa crowns for a week or longer. When the snow melted, aphids decreased to about the previous number. It is apparent that south-facing embankment temperatures were sufficiently high to allow a low population to survive the winter. These aphids probably served as a nucleus for buildup and reinfestation of surrounding fields during subsequent favorable conditions.

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The Flight Range of the European Chafer[1]

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ABSTRACT

With the use of fluorescent lacquer enamels a trap tree and an ultra-violet light, the flight habits of the European chafer, *Amphimallon majalis* (Raz.), were studied from 1954–56. Marked beetles were recaptured up to 725 yards on the night of their release. Since beetles average five mating flights in their lives, they could fly 4 to 5 miles in this time if they flew 725 yards in the same direction every evening and morning.

The single and seasonal flight ranges of insects are always of interest, but they are of utmost importance when dealing with economic pests occurring in localized areas under Federal and State quarantines. An underestimation of their seasonal flight range, or their normal spread, would mean that control and restrictive measures would fall short of desired goals while an overestimation would impose unnecessary economic hardships and restrictions on individuals.

Information was lacking on the single and seasonal flight ranges of the European chafer, *Amphimallon majalis* (Raz.), an important quarantined pest of meadows, pastures, winter grains, ornamental nurseries, and home lawns. Therefore, in 1954, in conjunction with studies on the influence of light on the nocturnal emergence of the European chafer (Evans & Gyrisco 1959) and orientation of flight, research was initiated on its flight ranges and was continued through 1956. This paper presents the results.

METHODS AND MATERIALS.—A considerable amount of literature is available on the dispersion and flight ranges of insects, particularly species of Diptera (Hocking 1953, Kettle 1951, Dobzhansky & Wright, 1943). In general, the procedure is to release marked insects at a central location and recapture them in various traps, or by sweeping at varying distances from the central source. Polivka (1949) released known numbers of Japanese beetles *Popillia japonica* New., marked with fluorescent lacquers, at a central location and periodically checked baited traps that had been set up at various distances in all directions from the release point. His conclusions on flight range of the Japanese beetles were based on recoveries of marked beetles found in the traps farthest from the release point. The methods used in these experiments may be considered as a reversal of the general procedure used by Polivka and other workers in that the recapturing site was centrally located while the release points were scattered at varying distances and directions from it.

The flight habits of the European chafer have been well described elsewhere (Schwardt & Whitcomb 1943, Gyrisco *et al.* 1954, Evans & Gyrisco 1959). In general, the beetles have crepuscular flights about silhouettes such as trees, shrubs, telephone poles, et cetera, to which they are attracted for the purpose of mating. Utilizing these facts, a tree, hereafter called the trap tree, located alone on a hill, was finally selected after several trials (Evans 1956, table 1) as the test collecting site. This tree, a conspicuous shagbark hickory about 25 feet tall, occupied a central position of a ridge in a treeline of smaller hickories which ran north and south. The nearest tree to the trap tree was about 60 yards away. To the east, the sod land sloped gradually downward for about 250 yards, then rose sharply to form a small hill and then gradually fell off again to a highway $1\frac{1}{2}$ miles away. To the west, the land was cultivated and rose gradually for about 100 yards before falling off gently to form a small depression then rose again to form another hill of about the same elevation as the trap tree. This rolling terrain continued for about 2 miles. Therefore, the trap tree dominated the gentle rolling countryside, which extended nearly 2 miles in each direction.

Each night during the flight season after the beetles had settled on the foliage of the trap tree, a 30′×30′ polyethylene sheet was placed on the ground around the tree so that it was directly below the overhanging branches. Then, starting from the top of the tree each branch was vigorously jarred, causing the beetles to fall on the sheet. (Beetles when disturbed will readily "play possum.") The large sheet was quickly gathered up and the beetles, unable to cling to the plastic, were easily maneuvered into one corner and were poured into a large can with a

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Table 1.—The numbers of European chafer marked with different colors and released at various distances from four trees and the percentage recaptured at each tree in preliminary trials. Wayne County, New York. June and July 1954.

No. Marked	Color Used	Direction and Distance in Yards from Tree	Recaptured (%)
	Tree #1—June 26		
684	Ark Yellow	Northwest 50	20.1
882	Light Green	Northwest 100	11.1
934	Light Orange	Northwest 150	2.5
1,416	Pearl White	Northwest 200	0.7
678	Satin Green	Southeast 50	13.2
790	Horizon Blue	Southeast 100	1.2
921	Dark Orange	Southeast 150	0.0
1,432	Purple	Southeast 200	0.0
	Tree #2—June 28		
164	Light Orange	Northeast 70	14.0
553	Light Green	Northeast 140	2.4
634	Pearl White	Northeast 210	1.5
772	Ark Yellow	Northeast 280	0.0
206	Purple	Southwest 70	2.6
450	Dark Orange	Southwest 140	0.0
650	Invisible Blue	Southwest 210	0.0
753	Satin Green	Southwest 280	0.0
	Tree #3—June 22		
232	Fire Orange	South×East 50	38.1
330	Neon Red	South×East 100	6.9
330	Signal Green	South×East 150	2.0
350	Invisible Blue	South×East 200	1.9
407	Ark Yellow	South×East 250	0.0
553	Satin Green	South×East 300	0.0
1,220	Pearl White	South×East 350	0.0
1,544	Horizon Blue	South×East 400	0.0
	Tree #3—July 2		
626	Light Green	South×East 100	11.5
915	Satin Green	South×East 200	1.3
1,833	Purple	South×East 300	0.2
663	Horizon Blue	North 100	24.8
1,258	Ark Yellow	North 200	0.6
1,793	Pearl White	North 300	0.0
632	Light Blue	Northeast 100	28.1
1,100	Light Green	Northeast 200	1.4
1,845	Fire Orange	Northeast 300	0.9

Table 2.—The numbers of recaptured, marked European chafer beetles after release from varying distances and directions from a centrally located trap tree. Wayne County, New York. June and July 1955.

Date Release of Marked Beetles	Distance to Trap Tree (Yards)	Direction of Release Site from Trap Tree	No. Beetles Marked (Estimated)	Days After Marking of any Recaptures	No. Marked Beetles Recaptured
June 19	150	East	2,079	1	10
				5	8
				9	7
				10	1
June 20	250	East	1,860	4	6
				8	2
June 23	250	East	5,174	1	24
				5	22
				6	6
June 25	1,830	Southwest	17,516	—	0
June 28	250	East	22,021	1	13
				3	4
June 29	525	East	14,646	—	0
July 1	300	East	7,390	2	5
				3	3
				4	2
				6	1
July 1	1,320	Northeast		—	0
July 3	725	East	11,679	1	1
				2	1
				6	1
July 4	300	East	9,794	1	12
				2	8
				3	3
				4	5
July 5	1,525	Southeast	5,320	—	0
July 6	350	East	2,152	1	2
				3	2
July 7	865	Southeast	4,779	—	0
July 8	400	East	3,200	1	3
				2	1
				3	1
July 9	625	East	9,721	1	1

screened lid. With a bit of practice the whole collecting operation took no more than 5 minutes.

The numbers of beetles taken were estimated by placing them in 1-quart cardboard ice cream containers and weighing them on a balance scale. A series of random samples indicated that there were 6.08 beetles per gram. On this basis subsequent collections of beetles were weighed and their numbers estimated.

Each evening during the flight season after the beetles were collected and their numbers estimated, they were marked with Glo-Craft Fuorescent Lacquer-Enamels obtained from Switzer Brothers, Cleveland, Ohio. Different colors were used to identify each lot of beetles and release station. The insects were marked by placing a handful at a time in the bottom of a large can and applying a fine spray with a small bulb-type atomizer of 1:1 mixture of enamel and acetone. The movements of the beetles in the can helped to distribute the marking mixture. Eventually all the beetles became marked with fine specks of the lacquer which soon became dry and adhered fast.

At varying measured distances and directions from the trap tree, release sites were established. These consisted of sections of 55-gallon drums buried in the soil or, in some cases, just previously dug shallow trenches. At the sites, the variously marked beetles were released and were covered lightly with soil and cages to prevent flight the day on which they were marked. On the following day, prior to any flight, the cages were removed. As shown in table 2, most release sites were located to the east of the trap tree because the trees' silhouette would be more prominent in the western sky at twilight.

Collections again were made at the trap tree during the same evenings following the release of marked beetles and nearly daily thereafter. These new catches were examined for marked specimens by placing several handfuls of beetles at a time in a shallow white enamel bowl over which was placed a glass plate. These were then exposed

to an ultra-violet light where the marked beetles fluoresced and were easily seen and removed. The re-collected marked beetles were saved for later comparisons with samples of marked beetles of all colors for exact identification of the color, and, thus, of the release site and date of release. In this manner it was possible to determine the numbers of beetles recaptured, distance of flight, the direction from which the beetles flew, and the period over which the insects flew. These data are given in table 2.

DISCUSSION.—It is quite evident from tables 1 and 2 that only a small number of the marked beetles was ever recovered. This result is as might be expected in a rolling, hilly, wooded countryside with abundant prominent silhouettes to attract and disperse the marked beetles. Recaptures of marked beetles fell off very rapidly at the trap tree from release sites more than 250 yards away, although preliminary trials at four other locations showed beetle recoveries were good up to 100 yards (see table 1).

Marked beetles were recovered up to distances of 725 yards on the night of their release. It is quite probable that the chafer flies greater distances in one evening, but owing to the attraction of other silhouettes in the experimental area, it is doubtful whether any marked beetles can be recovered from release points farther than 800 yards away. In wooded areas such as those found in central New York, the flight range of the European chafer is likely to be restricted because of the attraction of nearby trees to the flying beetles. As long as there are trees or similar silhouettes close enough to attract beetles after they emerge from the soil the adults tend to be confined to localized areas, thus restricting spread through flight.

The adults make an average of about five mating flights in their lifetime (Evans & Gyrisco 1959). If some flew 725 yards in the same direction every evening and again in the morning, they could fly, theoretically, 4 to 5 miles during their lives.

Some workers have speculated that there may be a tendency for flights in the evening to be made in a westerly direction since trees silhouetted on the western horizon would be very distinct and attractive compared with trees silhouetted in any other direction. When beetles are observed during their evening flight from the middle of a field, they appear to be flying in all directions. It is only near a tree or group of trees that any direction of flight can be noted, and that is toward the tree or groups. Experimental data (Evans 1956) on locations of new areas of infestations show no definite tendency for beetles to fly toward the west or any other direction (see table 1).

It would seem safe to suggest on the basis of these and other studies on flight (Evans 1956) that areas at a radius of 5 miles or more from the established periphery of a known infestation of European chafer need not be subjected to strict quarantine measures, since it is very unlikely that beetles spread naturally that far in a single flight season.

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Control of the Two-Spotted Spider Mite on Beans with Systemic Insecticides Applied in the Soil[1]

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ABSTRACT

Control of the two-spotted spider mite (*Tetranychus telarius* (L.)) on lima beans in southern California was obtained for 2 to 3 months by applying granulated or liquid insecticides in the seed furrow at planting time. Seed coatings were also effective. Phorate (formerly Thimet® (*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate)) and Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate) were used most extensively, but some of the other insecticides showed promise. Dosages of less than 1 pound per acre were as effective as higher dosages and showed no appreciable phytotoxicity. Crop yields were increased and little or no residue was found in the dry beans. Higher dosages resulted in reduced plant stands. For the control of thrips (*Frankliniella moultoni* Perg.), phorate was better than Di-Syston, but 2 to 4 pounds per acre were required.

Experiments were carried on from 1956 through 1958 in southern California on lima beans, to find out if systemic insecticides applied in the soil with the seed at planting time would control the two-spotted spider mite

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(*Tetranychus telarius* (L.)).[2] If such applications proved to be effective throughout the entire season, the expense of making a foliage application later on for mite control could be avoided. Since the western flower thrips (*Frankliniella moultoni* Perg.)[2] was present in the experimental fields, observations were also made on this insect, although it is not an important pest of lima beans.

METHODS.—The tests were conducted in fields of large lima beans grown for dry bean or seed production. Two- and four-row Ventura bean planters were used for planting the beans at about a 4-inch depth, and the insecticide applicators were mounted on the planters.

Granules were applied with the traction-powered applicator,[3] which meters them by the horizontal rotation of saw-tooth metal plates, the dosage being varied by the number of plates used. The granules were dropped into the planter boot by the use of flexible metal tubing. This applicator was limited to treatment of two rows at one time.

Liquids were applied through a spray nozzle attached to the back of the planter boot, and directed into the furrow as the seed was planted (Elmore 1950). In 1956 a traction-powered unit was operated satisfactorily at 7 gallons per acre. In 1957 a ¾-inch Oberdorfer gear pump was used, powered from the planter wheels by a V-belt, but it was not entirely satisfactory as the pressure was limited to about 20 pounds. In 1958 a 2-cylinder piston pump, powered by a gasoline motor, was used with a 4-row planter, and proved very satisfactory.

Seed coatings were applied as slurries prepared from 50% carbon mixtures. The fungicides thiram or captan were also used in the slurry. The beans were planted at about the normal rate of 100 pounds per acre. All experiments were set up in randomized blocks with six replicates, unless otherwise specified. At different intervals during the season the mites and thrips were counted on 10 half-leaflets per plot.

The materials tested and their effect on plant emergence and on mites are shown in table 1. All the insecticides were commercial formulations diluted to give the dosage indicated.

1956 Experiments.—In experiment A the plots were 100 feet long and one row wide (30 inches), with seven replicates, and planting was from May 21 to 23.

The beans emerging in each plot were counted on June 6 and 7. Reductions in stand resulted from all except two of the Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3 methyl) phosphorodithioate) and one of the Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate) treatments. However, all except the phorate (Thimet® (*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate)) seed treatment at 8 ounces per acre resulted in an average of one plant per foot of row, which usually produces a normal yield. At the rates used, the seed coatings of phorate were much more phytotoxic than either the liquid or granule applications.

The mite infestations came in late, and populations were low and erratic. Mite counts were made on July 16 and August 3 and 28. A significant degree of control (68% to 96%) was obtained with most of the materials used, but Guthion was consistently ineffective.

Thrips counts were made on June 19, July 5 and 16, and August 3 and 28. Results were good to excellent with the liquid and granule applications of phorate; fair with the phorate seed coatings, fair to good with Di-Syston, and poor with demeton, Chemagro 102 (*S*-2(ethylthio)ethyl *O,O*-dimethyl phosphorothioate), Chemagro 103 (*S*-2-(ethylsulfinyl)ethyl *O,O*-dimethyl phosphorothioate), and Chemagro 104 (*S*-2-(ethylsulfonyl)ethyl *O,O*-dimethyl phosphorothioate), and Guthion.

In experiment B the plots were 64 feet long and one row wide, and the seed was planted on May 30 and 31.

Plant stands were counted on June 18. Bayer 23129 (S-2-(ethylthio)ethyl *O,O* dimethyl phosphorodithioate) caused the least damage and was significantly better than phorate or Di-Syston. Guthion and Di-Syston caused less damage than phorate. The damage from phorate increased with the dosage. The phorate seed coatings were the most phytotoxic, as in experiment A.

Mite counts made on August 1 were too low and scattered to make significant comparisons.

Thrips counts were made on June 27, July 10, and August 1. When the 1- and 2-pound rates of each insecticide were averaged, the order of effectiveness was phorate, Di-Syston, Bayer 23129, and Guthion. When the phorate dosages of liquids and granules were averaged, the 4- and 2-pound dosages were better than the lower dosages. When liquid applications were compared with granules and averaged for all insecticides, no significant difference appeared.

1957 Experiments.—Experiment A was conducted in plots 100 feet long and 10 feet wide (four rows), and a round trip with the two-row planter was made in each plot. Planting was on April 11 to 13, and foliage sprays were applied on May 28.

Plant-emergence counts were made on May 3 on 80 feet of row per plot. The Di-Syston seed coatings all reduced the stands to less than one plant per foot of row, and the plots with the two higher strengths were replanted with untreated seed. In these plots the mites were controlled by the material remaining in the ground from the first planting. Demeton liquids and granules, used for the first time in this experiment, did not seriously affect the stand.

The mite populations did not build up until July. Counts made on July 19, 14 weeks after the soil treatment and 7 weeks after the foliage spray applications, showed a light but uniform infestation and significant control from all treatments. However, by the end of August the populations were higher in all the treated plots than in the untreated plots. This was owing to the fact that the high populations building up in the untreated plots damaged the plants so much that they became unattractive to the mites.

Thrips counts made on June 6, nine days after the foliage sprays and 8 weeks after planting, showed excellent control with the foliage sprays and fair to good control with some of the soil applications. In the averages of like treatments (excluding seed coating), phorate was better than Di-Syston and Di-Syston better than demeton. Foliage sprays were better than granules, and

[2] Mite determinations were made by E. A. McGregor, Entomology Research Division, U.S.D.A., Whittier, California, and thrips determinations by S. F. Bailey, University of California, Davis.

[3] Developed by Tobias Grether, of Oxnard, California.

Table 1.—Effect of systemic insecticides on bean plant emergence, and on populations of the two-spotted spider mite.

Formulation (Per Cent or Pounds per Gallon)	Toxicant (Oz./A.)	Plant Emergence (Per Cent of Check)	Spider Mites No. per Leaflet	Spider Mites Reduction (%)
Experiment A in 1956				
Soil Applications				
Phorate granules, 2%	15	83	0.5	96
Di-Syston granules, 2%	46	85	.7	95
Phorate EC, 4 lb.	9	86	.8	94
	20	78	.8	94
Chemagro 102 EC, 2 lb.	15	90	.9	93
Phorate in carbon, 50%[a]	4	55	1.0	92
Di-Syston granules, 2%	22	92	1.2	91
Chemagro 103, EC 2 lb.	15	98	1.2	91
Phorate in carbon, 50%[a]	8	25	1.4	90
Demeton EC, 2 lb.	15	99	1.4	89
Di-Syston EC, 2 lb.	15	106	1.8	83
Phorate granules, 2%	56	54	2.2	83
	33	62	2.4	81
Phorate EC, 4 lb.	38	66	4.2	68
Chemagro 104 EC, 2 lb.	15	106	7.4	44
Guthion granules, 5%	29	89	8.2	38
EC, 2 lb.	30	111	8.2	37
granules, 5%	51	91	8.4	36
Di-Syston EC, 2 lb.	30	89	8.4	36
Guthion EC, 2 lb.	15	116	11.5	12
Check	—	100	13.1	—
L.S.D. at 5% level		14	7.3	56
Experiment A in 1957				
Soil Applications				
Di-Syston EC, 2 lb.	13	92	3.6	95
granules, 2.5%	12	82	4.6	93
	13	79	5.3	92
Phorate granules, 2%	15	74	6.2	91
Di-Syston in carbon, 50%[a]	2	63	6.7	90
Phorate EC, 8 lb.	15	69	7.2	90
	8	101	7.8	89
Di-Syston EC, 2 lb.	7	96	8.0	89
Phorate granules, 2%[a]	7	85	9.5	87
Demeton granules, 2.5%	21	92	10.1	86
Di-Syston in carbon, 50%[a]	10	10	10.9	85
Demeton granules, 2.5%	3	100	12.7	82
EC, 2 lb.	14	93	13.6	81
Di-Syston in carbon, 50%[a]	8	20	14.2	80
Demeton EC, 2 lb.	7	88	21.0	70
Foliage Sprays				
Phorate EC, 8 lb.	17	—	2.7	96
Demeton EC, 2 lb.	9	—	3.8	95
Di-Syston EC, 2 lb.	9	—	5.9	92
Phorate EC, 8 lb.	9	—	7.2	90
Di-Syston EC, 2 lb.	4	—	13.9	80
Demeton EC, 2 lb.	4	—	20.1	72
Check	—	100	71.2	—
L.S.D. at 5% level		11	12.5	18
Experiment B in 1957				
Demeton granules 2.5%	16	109	0.4	98
Di-Syston granules 2.5%	33	97	.4	98
Demeton granules 2.5%	34	96	.9	97
EC, 2 lb.	12	104	1.1	96
Di-Syston granules 2.5%	17	108	1.1	96
Phorate EC, 8 lb.	14	91	1.4	95
	27	79	1.4	95
granules, 2%	14	104	1.5	95
Di-Syston EC, 2 lb.	12	91	1.6	94
Phorate granules, 2%	33	86	1.8	94
Demeton EC, 2 lb.	24	88	2.1	92
Di-Syston EC, 2 lb.	24	110	2.5	91
Guthion granules, 2.5%	15	109	20.6	26
	49	112	27.6	0
	36	105	38.1	0
Check	None	100	27.8	—
L.S.D. at 5% level		12	8.8	32
Experiment A in 1958				
Soil Applications				
Di-Syston granules 2.5%	22	104	6.0	94
Phorate EC, 8 lb.	38	58	6.6	94
granules 2%	29	89	10.8	90
Di-Syston EC, 2 lb.	12	100	11.2	89
	24	109	11.4	89
Phorate granules 2%	17	93	11.9	89
Di-Syston EC, 2 lb.	36	111	12.2	88
granules 2.5%	29	90	13.2	87
Di-Syston in carbon, 50%[a]	7	90	14.0	87
Phorate granules, 2%	7	96	15.7	85
Di-Syston in carbon, 50%[a]	2	96	16.0	85
granules, 2%	9	108	16.3	84
Phorate EC, 8 lb.	14	80	17.4	83
Di-Syston in carbon, 50%[a]	5	96	19.9	81
Phorate EC, 8 lb.	24	74	35.0	67
Bayer 29493 granules 1%	16	109	74.8	29
Bayer 25141 granules 1%	24	108	88.7	16
Bayer 29493 granules 1%	25	119	93.5	11
Bayer 25141 granules 1%	5	104	95.9	9
Bayer 29493 granules 1%	5	107	103.4	2
Bayer 25141 granules 1%	14	100	110.5	0
Foliage Sprays				
Phorate EC, 8 lb.	13	—	27.8	74
Demeton EC, 2 lb.	7	—	28.5	73
Check	—	100	105.5	—
L.S.D. at 5% level		17	29.0	27
Experiment B in 1958				
Soil Applications				
Di-Syston EC, 2 lb.	12	107	3.5	94
Phorate EC, 8 lb.	26	91	3.6	94
Di-Syston EC, 2 lb.	23	108	4.2	93
Phorate EC, 8 lb.	14	102	5.3	92
Di-Syston EC, 2 lb.	6	111	6.7	89
Am. Cyanamid 18706 EC, 2 lb.	19	21	7.1	89
Phorate EC, 8 lb.	6	100	7.5	88
Dimethoate EC, 4 lb.	10	99	9.4	85
	19	71	11.4	82
Am. Cyanamid 18706 EC, 2 lb.	10	76	19.7	69
Monsanto 10502 EC, 2 lb.	23	99	41.4	35
	12	98	42.0	34
Monsanto 8574 EC, 1 lb.	12	96	44.0	31
	6	106	50.4	20
Foliage Sprays				
Am. Cyanamid 18706 EC, 2 lb.	7	—	2.2	97
Demeton EC, 2 lbs.	7	—	3.7	94
Dimethoate EC, 4 lb.	10	—	3.8	94
Monsanto 8574 EC, 1 lb.	8	—	6.1	90
Phorate EC, 8 lbs.	11	—	7.4	88
	7	—	8.3	87
Demeton EC, 2 lb.	4	—	9.6	85
Dimethoate EC, 4 lb.	5	—	9.7	85
Am. Cyanamid 18706 EC, 2 lb.	3	—	10.0	84
Phorate EC, 8 lb.	5	—	11.3	82
Monsanto 8574 EC, 1 lb.	3	—	14.7	77
Monsanto 10502 EC, 2 lb.	4	—	41.1	35
	8	—	51.5	19
Check	—	100	63.4	—
L.S.D. at 5% level	—	12	16.0	25

[a] Seed treatment, slurry with thiram.

granules better than liquids. The 1-pound dosage tended to be better than the ½-pound.

In experiment B planting and soil treatments were made on May 9 and 10. Plant emergence was excellent, and even the lowest averaged 1.8 plants per foot of row. However, the plant stands with the granules were better than those with liquids, and demeton and Di-Syston were better than phorate. Guthion, used only in granules, had a little better stand than granules of the other materials.

Mite populations were very low in the first two counts. On July 29 all materials except Guthion showed excellent control of low populations. By September 3, owing to the light infestation, no difference was apparent between treatments.

Thrips counts, made on June 12, July 5 and 29, and September 3, showed a significant but only fair to poor control over this 16-week period. On June 12 phorate showed nearly 100% control and was better than Di-Syston or demeton. Guthion (granules only) showed little control of thrips at any time.

1958 Experiments.—Experiment A was conducted in plots 4 rows wide and 100 feet long. Planting and soil treatments were made May 12 to 14. Two sets of plots were reserved for foliage applications made on June 19.

Plant stands were checked on June 2 by counting 40 feet of row in each plot (20 feet from each of the two center rows). Significant reductions in stand were ap-

Table 2.—Insecticide residues in dry lima beans and yields after applications of systemics in experiment A, 1958.

INSECTICIDE	AVERAGE OUNCES OF TOXICANT PER ACRE	RESIDUE[a] (P.P.M.)	AVERAGE YIELD PER ACRE (100-LB. SACKS)
Soil Treatments			
Di-Syston			
Liquids	24	0.1	22.8
Seed coatings	5	0	22.2
Granules	20	0.1	21.6
Phorate			
Granules	18	0[b]	21.0
Liquids	25	0	20.0
Bayer 25141, granules	14	—	16.2
Bayer 29493, granules	15	—	16.0
Foliage Sprays			
Phorate	13	0	22.9
Demeton	7	—	20.0
Check	—	—	15.4
L.S.D. at 5% level	—	—	1.7

[a] Di-Syston analyses made by W. E. Westlake of the Entomology Research Division, and phorate analyses by Mason H. Woolford, Jr., American Cyanamid Co.

[b] In this table, 0 residue of phorate means less than 0.008 p.p.m.

parent only from the liquid soil applications of phorate.

Mite counts were made on June 30, July 15 and 30, and August 18. All treatments show a significant degree of control except Bayer 25141 (*O,O*-diethyl *O*-(*p*-methylsulfinyl)phenyl phosphorothioate) and Bayer 29493 (*O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate). The low dosages were just as good as the medium or high dosages.

Crop yields were taken by cutting the middle 50 feet of the two center rows in each plot. Normal yields of 20 to 23 100-pound sacks of dry beans per acre were obtained with all treatments that gave significant mite control, as compared with 15 to 16 sacks in the check plots and with Bayer 25141 and 29493 (table 2).

Analyses of shelled dry beans from the Di-Syston plots showed 0 to 0.1 p.p.m. of Di-Syston residues by the cholinesterase inhibition method. Analyses of comparable samples from the phorate plots, using the same method, showed no phorate residues.

One thrips count was made on June 30, eleven days after the foliage sprays and 45 days after the soil treatments. Phorate and demeton foliage sprays gave excellent control, and control from soil applications was in relation to the amount of insecticide applied. Phorate was better than Di-Syston. The seed treatments of Di-Syston and the soil applications of Bayer 25141 and 29493 were ineffective. No further thrips counts were made, because DDT plus toxaphene for lygus bug control was applied over the entire field on June 30 and July 30, and these applications kept the thrips populations at a very low level.

In experiment B the plots were 100 feet long and eight rows wide. On May 20 to 23 about half the field was planted and treated with the liquid insecticide. Foliage sprays were applied to the remainder of the plots on June 23 to 26.

Plant emergence was checked on June 9. All dosages of phorate and Di-Syston gave a normal stand and Monsanto 8574 (tetramethyl(dithiodimethylene)diphosphonate) and Monsanto 10502 (1-(dimethoxyphosphinyl)-vinyl dimethyl phosphate) caused no damage. Dimethoate (at the high dosage) and Am. Cyanamid 18706 (*S*-(*N*-ethylcarbamoylmethyl) *O,O*-dimethyl phosphorodithioate) (at both dosages) caused a reduction in stand, and the plots receiving the high dosage had to be replanted.

Mite populations were counted July 8 and 21 and August 4 and 20. Counts from the soil applications over a 3-month period compared very favorably with the foliage spray counts over a 2-month period, although the spray applications were at lower dosages. In the soil treatments phorate, Di-Syston, dimethoate, and Am. Cyanamid 18706 gave good to excellent control, while Monsanto 8574 and Monsanto 10502 were poor. In the foliage sprays, demeton was used instead of Di-Syston, and results were similar to those obtained with the soil treatments except that Monsanto 8574 gave good results, while Monsanto 10502 was poor.

Yields were not taken in this field because the alkali spots would have caused more variation than the treatments in results.

Thrips populations were counted only on July 8 and 21, because all the plots were sprayed with DDT for lygus bug control. No significant control was evident from the soil applications in these counts. Foliage spray counts made 2 to 4 weeks after application were especially promising with dimethoate and Am. Cyanamid 18706, but demeton and phorate were only fair, and Monsanto 8574 and Monsanto 10502 did not give significant control of thrips.

DISCUSSION.—These experiments indicate that satisfactory control of the two-spotted spider mite on lima beans can be accomplished by applying systemic insecticides on the seed, or in granulated or liquid form in the soil with the seed at planting. Most of the tests so far have been made under light or late infestations, and show no superiority of this method over foliage applications made 4 to 8 weeks after planting. Phorate and Di-Syston showed as high as 95% control of mites for 3 months after planting. Dosages of 2 or 4 pounds per acre reduced the stands in many tests. Indications are that one-half pound or less can be used with good results and with little damage to the stand.

In southern California it is often necessary to make foliage applications for control of lygus bugs at or near blossoming, about July 1. Under late infestations of the two-spotted spider mite such as have prevailed for the last 3 years, it may be more practical to make one foliage application for control of both mites and lygus. However, lima bean seed is always coated with a fungicide in a slurry with dieldrin or lindane for control of rot, wireworms, and the seed-corn maggot (*Hylemya cilicrura* (Rond.)), at a cost of about 75 cents per acre; so a systemic material for control of mites can be added to the seed coating with only a slight increase in cost. Even if an early infestation of mites occurred only once in 5 years, it would be profitable to treat the seed every year in order to protect the plants during the early stages.

REFERENCE CITED

Elmore, John C. 1950. A device for spraying bean seed in the row while planting. U. S. Bur. Ent. and Plant Quar. **ET-279.**

Control of Boll Weevils Resistant to Chlorinated Hydrocarbons[1]

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Abstract

During 1956, 1957, and 1958 15 replicated field-plot insecticide-comparison experiments were conducted in the Robertson-Burleson County area of south-central Texas to determine the effectiveness of various insecticides for control of boll weevils, *Anthonomus grandis* Boh., resistant to chlorinated hydrocarbon insecticides. Guthion® (*O,O*-dimethyl *S*-(4oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate), Sevin® (1-naphthyl methylcarbamate), and calcium arsenate dust were most effective in controlling these weevil populations. Methyl parathion and malathion effected good immediate kill of boll weevils but did not always prevent excessive weevil damage when applied at the customary 5-day interval. Chlorinated hydrocarbon materials varied considerably in relative effectiveness at the various locations but were generally the least effective of the insecticides used. All insecticides except Sevin were combined with DDT for bollworm control.

Farmers were unable to control the boll weevil, *Anthonomus grandis* Boh., with chlorinated hydrocarbons in a large area of Louisiana during 1955. Workers in that State demonstrated that the failure was due to resistance of these insects to the chlorinated hydrocarbon insecticides (Roussel & Clower, 1955).

In July 1956 cotton planters in a small area of Robertson County, Texas, were unable to control the boll weevil with regularly scheduled applications of the chlorinated hydrocarbon insecticides. Irrigated cotton fields bordering the densely wooded Little Brazos River seemed to be most heavily infested. Laboratory toxicity tests on boll weevils from this area indicated a high degree of resistance to the chlorinated hydrocarbon materials (Walker *et al.* 1956). Farmers controlled these severe boll weevil infestations with phosphate insecticides or with mixtures of phosphates and chlorinated hydrocarbons.

In this portion of Robertson County during 1957, cotton planters were unable to obtain satisfactory control of overwintered populations of the boll weevil on seedling cotton with chlorinated hydrocarbons, but phosphate insecticides were effective. During 1956 and 1957 few cases of control difficulties with chlorinated hydrocarbons in adjacent Burleson County were reported. Although reports from Burleson County during 1958 indicated that chlorinated hydrocarbon insecticides had failed to control the weevil, it was difficult to appraise the situation accurately because at the first indication of control difficulties growers would change to phosphate materials or mixtures of phosphates and chlorinated hydrocarbons. Consequently, no widespread failures in boll weevil control appeared in Burleson County such as occurred in the Little Brazos area in 1956.

During 1956, 1957, and 1958 15 replicated field-plot insecticide-comparison experiments were conducted in the Robertson-Burleson County area to determine the effectiveness of various insecticides for control of boll weevils resistant to chlorinated hydrocarbons and to obtain some idea of practical variations in resistance in this area.

Materials and Methods.—During 1956 experiments 1, 2, 3, 4, and 5 were conducted in Robertson County. Tests were located in irrigated cotton fields where boll weevil infestations had not been adequately controlled with applications of chlorinated hydrocarbon insecticides. During 1957 experiments 6, 7, 9, and 10 were conducted in irrigated cotton fields in Robertson County, while experiment 8 was located in Burleson County in a nonirrigated cotton field. The boll weevil infestations during 1957 were not so severe as those encountered during 1956. During 1958 all tests were located in fields in Burleson County. One of these, experiment 12, was situated in an irrigated field. Experiments 11, 13, 14, and 15 were located in nonirrigated fields. Only moderate infestations of boll weevils occurred during the 1958 season.

Sprays were applied at the rate of 7.5 gallons per acre during 1956 and 1957 and at a rate of 5 gallons per acre during 1958. The experimental calcium arsenate-DDT spray formulation used during the 1958 season was applied at the rate of 10 gallons per acre. The sprays were applied with a self-propelled row-crop sprayer whenever the cotton fields were dry. Hand sprayers were used during periods of irrigation or rain. All insecticidal dusts were applied with hand guns at approximately 15 pounds per acre. All insecticide applications and insect-infestation records were made at about 5-day intervals during the boll weevil and bollworm infestation period.

The field design, plot sizes, and dosages of insecticides for each experiment are shown in tables 1 and 2. Experiments 12 and 14 each involved 11 treatments. In order to obtain better control of soil and infestation variation among the numerous plots, a Youden square design was employed. The insecticides in most tests were compared by using percentages of punctured squares as the criterion of effectiveness. After an analysis of variance, the significant differences among treatment means were determined by the use of Duncan's multiple range test. In experiment 15 records of both live overwintered weevils and punctured squares were used as criteria for comparing the treatments' effectiveness. The overwintered weevil phase of experiment 15 was conducted in the following manner: In each of the 2-acre plots, a record was taken of the number of live weevils present on 200 feet of row. Each weevil found before treatment was marked with a spot of lacquer. The insecticides were applied immediately after this count was made. Subsequent counts of the marked and unmarked weevils present on the previously sampled sections of row at intervals were made after each treatment. Four applications of the insecticides and 10 weevil counts were made during the study. On the date of the last live-weevil count a punctured square record was made.

The late-season control phase of experiment 15 was initiated at the time of emergence of the first generation

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Table 1.—Control of late-season boll weevil infestations.

Treatment	Pounds Active Ingredient per Acre	Per Cent Punctured Squares[a]
Experiment 1—1956. Randomized block, 0.08-acre plots, 4 replications. Eight spray applications from 7/9 to 8/16		
Aldrin-DDT	0.39–0.97	75.9
BHC gamma-DDT	0.51–0.97	76.7
Dieldrin-DDT	0.39–0.98	77.3
Heptachlor-DDT	0.41–1.01	77.4
Thiodan[b]	0.37	81.5
Check	—	83.9
Experiment 2—1956. Randomized block, 0.1-acre plots, 4 replications. Eight spray applications from 7/19 to 8/30		
Guthion-DDT	0.41–0.62	38.2
Methyl para.-toxaphene-DDT	0.55–2.20–1.10	40.9
Methyl para.-DDT	0.65–0.90	51.3
Dieldrin-DDT	0.39–0.90	54.8
Check	— —	59.0
Experiment 3—1956. Randomized block, 0.1-acre plots, 4 replications. Five dust applications from 7/20 to 8/11		
Calcium arsenate	10.36	22.1
Calcium arsenate-DDT	11.92–0.45	23.1
Guthion-DDT	0.38–1.53	26.3
Malathion-DDT	1.23–1.54	30.0
Thiodan	0.50	37.5
Dieldrin-DDT	0.38–1.52	37.8
Heptachlor-DDT	0.39–1.55	39.6
Check	—	46.3
Experiment 4—1956. Randomized block, 0.10-acre plots, 4 replications. Fourteen spray applications from 7/12 to 9/18		
Guthion-DDT	0.46–0.67	61.6
Methyl para.-DDT	0.75–1.00	63.6
Malathion-DDT	1.54–1.10	64.8
Toxaphene-DDT	2.60–1.30	66.4
Phosdrin[c]-DDT	0.36–0.96	76.1
Check	— —	82.6

Experiment 5—1956. Split plot. Plot size 0.1 acre, 4 replications. Twelve applications from 7/12 to 9/13

				Dust and Spray Averages
Guthion-DDT	spray	0.42–0.63	58.1	50.6
	dust	0.48–0.93	43.0	
Methyl para.-DDT	spray	0.72–1.07	59.8	62.8
	dust	0.68–1.32	65.9	
Toxaphene-DDT	spray	2.46–1.23	70.6	73.8
	dust	3.22–1.61	77.0	
Endrin	spray	0.43	77.5	78.4
	dust	0.30	79.2	
Check	—		82.2	81.8
	—		81.3	

Experiment 6—1957. Randomized block, 0.10-acre plots 4 replications. Ten spray applications from 7/8 to 8/21		
Malathion-toxaphene-DDT	0.50–0.99–0.99	37.9
Malathion-DDT	1.26–1.01	44.6
Methyl para.-heptachlor-DDT	0.50–0.38–1.07	46.5
Methyl para.-DDT	0.62–1.02	47.2
Toxaphene-DDT	2.00–1.00	58.5
Check	— —	66.0

[a] Insecticides arrayed in order of effectiveness; means included in same brackets not significantly different.

[b] 6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*, hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide.

[c] 1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical.

Table 1.—(Continued)

Treatment	Pounds Active Ingredient per Acre	Per Cent Punctured Squares[a]
Experiment 7—1957. Randomized block, 0.10-acre plots, 4 replications. Fifteen spray applications from 7/11 to 9/11		
Guthion-DDT	0.26–1.02	29.4
Methyl para.-endrin	0.45–0.25	52.6
Toxaphene-DDT	2.00–1.00	52.8
Diazinon[d]-toxaphene-DDT	0.43–1.08–1.08	54.7
Check	— —	67.6
Diazinon-DDT	0.44–1.00	70.0
Experiment 8—1957. Randomized block, 0.1-acre plots, 4 replications. Ten spray applications from 7/8 to 8/21		
Guthion-DDT	0.25–1.00	14.7
Methyl para.-DDT	0.4 –1.00	18.7
Toxaphene-DDT	2.00–1.00	24.3
Malathion-DDT	1.26–1.02	24.7
Thiodan	0.25	39.8
Thiodan	0.49	46.0
Check	—	65.5
Experiment 9—1957. Randomized block, 0.10-acre plots, 4 replications. Eight dust applications from 7/15 to 9.2		
Sevin	1.65	9.0
Calcium arsenate-endrin	11.10–0.32	11.5
Calcium arsenate-parathion-DDT	9.80–0.14–0.73	13.1
Toxaphene-DDT	2.30–1.15	28.5
Check	— —	29.7
Experiment 10—1958. Randomized block, 0.1-acre plots, 4 replications. Ten spray applications from 7/26 to 9/11		
Compound 7769-DDT	0.42–0.85	21.8
Dicapthon-DDT	1.05–1.05	24.9
Methyl para.-endrin DDT	0.52–0.24–0.48	34.6
Dieldrin-DDT	0.26–1.03	39.4
Thiodan	1.35	52.4
Check	— —	60.9
Experiment 11—1958. Randomized block, 0.1-acre plots, 3 replications. Eight applications from 7/2 to 8/14		
Sevin-dust	1.57	7.0
Dicapthon-DDT spray	1.06–1.06	11.4
Sevin-dust	0.80	12.7
Dicapthon-DDT spray	0.50–1.00	17.9
Compound 7769-DDT spray	0.49–0.98	18.6
Compound 7769-DDT spray	0.25–1.00	26.8
Toxaphene-DDT spray	1.97–0.98	27.3
Check	— —	33.1
Experiment 12—1958. Youden square, 0.1- acre plots, 5 replications. Six applications from 7/31 to 8/20		
Sevin-dust	1.48	5.5
Toxaphene-methyl para.-DDT spray	1.96–0.46–0.98	7.8
Sevin suspension-spray	1.49	8.9
Methyl para.-DDT spray	0.52–1.04	11.9
Dicapthon-DDT spray	1.06–1.06	12.2
Guthion-DDT spray	0.25–1.00	13.4
Toxaphene-DDT spray	2.01–1.01	16.1
Heptachlor-DDT spray	0.5 –1.00	20.9
Dieldrin-DDT spray	0.35–1.05	25.4
DDT	1.04	29.5
Check	—	32.2
Experiment 13—1958. Randomized block, 0.1-acre plots, 4 replications. Seven applications from 7/17 to 8/19		
Calcium arsenate-DDT dust	12.7 –1.60	23.0
Toxaphene-DDT spray	1.9 –0.95	36.0
Check	—	45.0
Calcium arsenate DDT suspension spray	10.40–1.60	50.0

[d] *O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate.

Table 1.—(Continued)

Treatment	Pounds Active Ingredient per Acre	Per Cent Punctured Squares[a]
Experiment 14—1958. Youden square, 5 replications, 0.1 acre plots. Seven applications from 7/4 to 8/14		
Sevin-dust	1.51	5.6
Guthion-DDT spray	0.24–0.96	8.1
Sevin suspension-spray	1.48	10.0
Toxaphene-methyl para.-DDT spray	1.95–0.48–0.97	10.7
Methyl para.-DDT spray	0.50–1.00	12.1
Dicapthon-DDT spray	1.04–1.04	12.7
Dieldrin-DDT spray	0.33–1.00	13.5
Toxaphene-DDT spray	2.05–1.02	15.3
DDT spray	1.05	15.5
Heptachlor-DDT spray	0.48–0.96	16.0
Check	—	16.1

[a] Insecticides arrayed in order of effectiveness; means included in same brackets not significantly different.

of boll weevils. The percentages of punctured squares on five different dates were averaged to compare the effectiveness of the insecticides during this period.

RESULTS.—The chlorinated hydrocarbon insecticides were found to be ineffective for boll weevil control in field experiments conducted in Robertson County during 1956. In some tests these compounds reduced the infestation below that of the check but these reductions were never great enough for economic control of the pest. In three of the experiments conducted, Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate) DDT exhibited considerable effectiveness in reducing boll weevil infestations despite the fact that the emulsion concentrate formulation was rather poor. Malthion and methyl parathion reduced the punctured square infestations but were not highly effective at the treatment intervals maintained. In one experiment a mixture of toxaphene-methyl parathion-DDT was apparently very effective. Calcium arsenate dust was used in one test where the infestation was moderate, and resulted in good boll weevil control.

The use of chlorinated hydrocarbons in experiments 6, 7, 9, and 10 (conducted in the Robertson County area during 1957) resulted in little or no control of the boll weevil. Guthion, dicapthon, Compound 7769 (hexaethyl (ethylthiomethylidine) triphosphonate), calcium arsenate, and Sevin® (1-naphthyl methylcarbamate) were found to be very effective. Malathion and methyl parathion were moderately effective. In experiment 7, a mixture of methyl parathion-endrin failed to reduce the infestation significantly. Applications of toxaphene-DDT in experiment 8 in the Burleson County area resulted in very good control of the boll weevil.

Late-season infestations of boll weevils were rather light in the five tests conducted during 1958. In experiments 11, 12, 13, and 14 the use of the chlorinated hydrocarbons resulted in poor boll weevil control while most phosphates, calcium arsenate dust, and Sevin gave good reductions of the weevil populations. Although Compound 7769 appeared to be a promising material in 1957, it did not appear so effective in one test in which it was included during 1958. An experimental suspension spray formulation of calcium arsenate-DDT was relatively ineffective.

In experiment 15 it was apparent from the live-weevil records and the averages of percentage punctured squares that toxaphene was less effective than malathion or Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) for the control of overwintered boll weevils. However, 1 pound of malathion or Trithion per acre was required for effective control of overwintered weevils. When DDT was added to all treatments during the late-season phase of this test, the effectiveness of all three mixtures was about equal.

DISCUSSION.—The use of the chlorinated hydrocarbons in most of the tests conducted during 1956, 1957, and 1958 resulted in some reduction of boll weevil populations. This reduction in most cases did not provide satisfactory control. The effectiveness of the chlorinated hydrocarbons appeared to be influenced by the variations in boll weevil resistance within the area and by the degree of severity of the infestations. In fields where great numbers of weevils were present at the beginning of a control program, a highly effective material was required to reduce the infestation. Where fewer numbers were present, less effective materials gave reasonable control.

Applications of Guthion, Sevin, and calcium arsenate at 5-day intervals were found to be highly effective for boll weevil control; weevils in severely infested fields were brought under control without reducing the 5-day application interval. With malathion and methyl parathion

Table 2.—Control of early- and late-season boll weevil infestations. 1958.

Item	Toxaphene	Malathion	Trithion
Experiment 15[a]			
Overwintered Boll Weevil Control			
Toxicant applied 5/14[b]	1.0	0.5	0.3
5/22	1.0	.5	.3
Weevils on 200 feet of row 6/9	14.3	8.3	16.3
Toxicants applied 6/9[b]	2.0	.5	.5
Weevils on 200 feet of row 6/11	10.3	3.7	7.3
% weevils marked on 6/9 recovered 6/11	33.6	7.5	20.4
Weevils on 200 feet of row 6/13	14.0	4.3	6.3
% weevils marked on 6/9 recovered 6/13	24.3	10.3	7.7
Weevils on 200 feet of row 6/17	13.0	5.7	7.7
Weevils marked on 200 feet or row 6/19[c]	19.7	19.0	19.3
Toxicants applied 6/19[b]	3.0	1.0	1.0
Weevils on 200 feet of row 6/20	14.3	.7	.7
% weevils marked on 6/19 recovered 6/20	14.3	.0	.7
Weevils on 200 feet of row 6/23	5.0	.3	1.0
% weevils marked on 6/19 recovered 6/23	6.7	.0	.0
% punctured squares (by overwintered weevils) 6/23	39.5	20.3	16.2
Late-Season Boll Weevil Control	Toxaphene-DDT	Malathion-DDT	Trithion-DDT
Average amounts of toxicant applied (9 applications 6/24–8/2[b]	2.0–1.0	1.0–1.0	1.0–1.0
Average % punctured squares 7/2–7/22	23	25	18

[a] Randomized block, 2-acre plots, 3 replications.
[b] Pounds active ingredients per acre applied as sprays.
[c] Weevils from untreated area added to same plots to equalize numbers.

under the same conditions more frequent applications were needed for effective control.

In some cases mixtures of phosphate and chlorinated hydrocarbon materials appeared to be more effective than phosphates used alone. The relative effectiveness of these mixtures as well as the chlorinated hydrocarbon combinations containing DDT may have been influenced by the degree of chlorinated hydrocarbon resistance in local populations.

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Methylenedioxyphenyl Derivatives as Synergists for Carbamate Insecticides on Susceptible, DDT-, and Parathion-resistant House Flies[1]

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ABSTRACT

2-(2-ethoxyethoxy)ethyl 3,4-methylenedioxyphenyl acetal of acetaldehyde (sesamex) and 3,4-methylenedioxyphenyl benzenesulfonate were found to be active synergists for four carbamate insecticides against susceptible, DDT-, and parathion-resistant house flies (*Musca domestica* L.). In contrast to the usual ratios of synergist to insecticide, in this study, insecticide/synergist ratios higher than 1.0 were used. Cross resistance and levels of tolerance to the carbamate insecticides by DDT- and parathion-resistant flies were determined. Studies of the *in vitro* inhibition of human blood plasma and fly head cholinesterases have shown that with the exception of Sevin® (1-naphthyl-N-methyl carbamate), the carbamates studied are less active inhibitors for fly head cholinesterases than for human plasma cholinesterases.

The discovery of compounds which enhance the effectiveness of pyrethrins in fly sprays has provided a stimulus for intensive research of synergism in insecticides. Since Pearson's (1935) discovery that pine oil increased the efficiency of pyrethrum cattle sprays and Eagleson's (1940) finding that sesame oil promoted the insecticidal effect of pyrethrin solution for house flies (*Musca domestica* L.), Haller *et al.* (1942) have isolated the active ingredient, sesamin. Wachs (1947) introduced the compounds piperonyl cyclonene and piperonyl butoxide which have since been used in commercial fly sprays. Beroza (1956) discovered that several other derivatives of 3,4-methylenedioxyphenol (sesamol) are active synergists for pyrethrum. Several of these synergists were tested for synergism with different groups of insecticides and showed different degrees of activity. Perry *et al.* (1951) found that piperonyl cyclonene synergizes DDT on DDT-resistant flies. Also some of these compounds have shown synergism with organophosphorus insecticides (Hoffman *et al.* 1954). Moorefield (1958) reported that some carbamate insectides can be synergized with pyrethrin synergists. We have reported (Eldefrawi *et al.* 1959) that sesamex (a pyrethrin synergist) activates Sevin® (1-naphthyl-N-methyl carbamate) against susceptible and resistant flies.

However, with all the knowledge accumulated in the literature about insecticide activation by synergists, only pyrethrin-synergist combinations have found practical use. Since these synergists have been used in 5 to 10 times the concentrations of the insecticide and since they are more expensive than most of the insecticides, their extensive use has not been economically feasible at these ratios. Therefore, to combine scientific value with practical value with practical use, compounds are needed that will activate the insecticides at very low concentrations. We worked along these lines and have found that some of the 3,4-methylenedioxyphenyl derivatives are active synergists for a group of carbamate insecticides at high insecticide to synergist ratios.

MATERIALS AND METHODS.—Four carbamate insecticides were used in this study. Sevin was obtained from Union Carbide Chemicals Co., Dimetilan (3-methyl-5-pyrazolyl dimethylcarbamate), Pyrolan® (5-(3-methyl-1-phenylpyrazolyl) dimethylcarbamate) and Isolan, (5-(3 methyl-1-isopropylpyrazolyl) dimethyl carbamate were obtained from Geigy Chemical Co. Sesamex and sesamol were obtained from Shulton, Inc. The vinyl ethers used to synthesize the acetal derivatives of sesamol were supplied by Union Carbide Chemicals Co. and the synthesis was carried out as described by Beroza (1956).

The house flies used for the study were as follows: the susceptible strain had been bred in the laboratory for several years without exposure to any insecticide. The DDT-resistant strain was obtained originally from Stauffer Agricultural Research Laboratory, Los Altos, California, and kept under DDT pressure. The parathion-resistant strain was obtained from the University of California, Citrus Experiment Station, Riverside, California. The latter strain has been reared for 8 months without further exposure to insecticides and still shows 10-fold resistance to parathion.

Twenty-five 4 to 6-day-old female house flies were used in these experiments. Acetone solutions of insecticides and synergists were applied by a micrometer driven syringe delivering 1 microliter per fly and by a 1-microliter micropipette. The solutions were applied simultaneously to CO_2 anesthetized flies and all applications were on the notum. Controls were treated with acetone only. Neither synergist gave any kill at the highest concentrations

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Table 1.—The effect of two synergists upon mortality from Sevin; 1 μg./fly for susceptible and DDT-resistant and 2 μg./fly for parathion-resistant.

Strain of House Fly	Per Cent Mortality at 24 Hours						
		+Sesamex (μg.)			+Benzenesulfonate Ester (μg.)		
	Sevin	0.1	0.2	0.5	0.1	0.2	0.5
Susceptible	10[a]	76	96	100	100	96	100
DDT-resistant	0.0	70	85	100	—	—	—
Parathion-resistant[b]	0.0	—	12	48	—	36	76

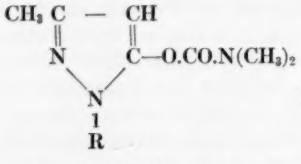
[a] Numbers below the double line are mortality percentages.
[b] When synergists applied at the level of 1 μg./fly plus 2 μg. Sevin both gave over 90% kill.

used. The treated flies were fed sugar and water, and mortality counts were taken after 24 hours.

Results.—Four acetal derivatives of sesamol: 2-(2-ethoxyethoxy) ethyl 3,4-methylenedioxyphenyl acetal of acetaldehyde; 2-(2-chloroethoxy)ethyl 3,4-methylenedioxyphenyl acetal of acetaldehyde; 2-butoxyethyl 3,4-methylenedioxyphenyl acetal of acetaldehyde; 2-(2-butoxyethoxy)ethyl 3,4-methylenedioxyphenyl acetal of acetaldehyde and the benzenesulfonic acid ester of sesamol were tested for synergism with Sevin using 5 to 1 and 10 to 1 insecticide to synergist ratios. Only the 2-(2-ethoxy-ethoxy)ethyl 3,4-methylenedioxyphenyl acetal of acetaldehyde (sesamex) and the benzenesulfonic acid ester of sesamol showed high synergistic activity.

The LD_{50} values (μg./fly) for DDT and parathion on DDT- and parathion-resistant flies were 18 and 0.07, respectively, compared with 0.2 and 0.0076 on susceptible flies. In other words, the DDT-resistant flies exhibit 90-fold resistance to DDT and the parathion-resistant flies about 10-fold. The latter also showed different levels of cross resistance to several chlorinated hydrocarbons March & Prenty (1959).

Sevin was not very effective as an insecticide on susceptible flies, LD_{50} being 2.60 μg. as compared with 0.20 μg. for DDT and 0.0076 μg. for parathion. The DDT-resistant flies exhibited a 10-fold increase in LD_{50} value toward Sevin which can be regarded as moderate cross resistance (fig. 1). The parathion-resistant flies showed high cross resistance to Sevin since a dosage of 20 times the LD_{50} for susceptible flies gave only 10% mortality on the parathion resistant. Application of the two test synergists sesamex, or 3-4 methylenedioxyphenyl benzenesulfonate, at high Sevin/synergist ratios showed a high degree of activation against the three test strains (table 1).



R (I=H, II=iso-Propyl, III =phenyl)

The three Geigy carbamates, Dimetilan (I), Isolan® (II), and Pyrolan (III) showed different levels of toxicity to house flies but all were more effective than Sevin. Figure 2 shows the log dosage-probit (ld-p) lines for Dimetilan on the three strains of flies. Dimetilan has almost the same activity on both susceptible and DDT-resistant strains, but the parathion-resistant strain

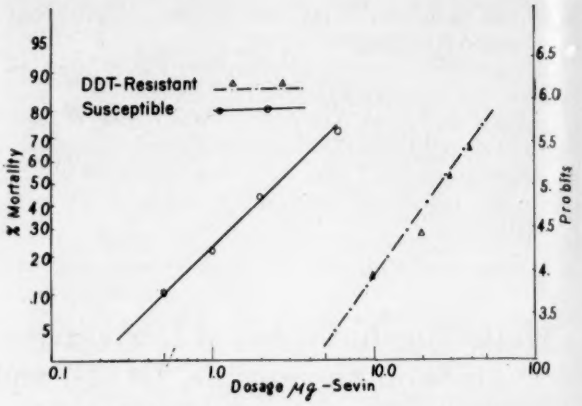


Fig. 1.—Probit-log dosage lines for two strains of house flies; Sevin in acetone applied to the notum.

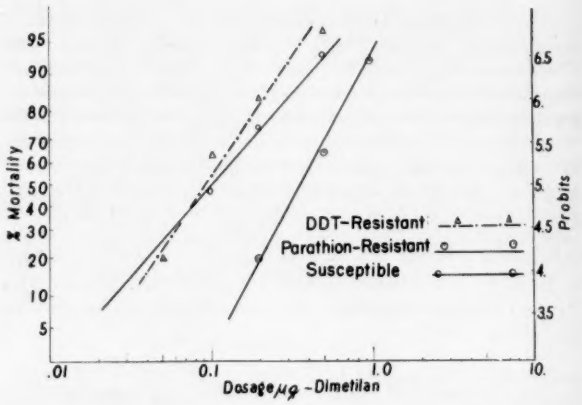


Fig. 2.—Probit-log dosage lines for three strains of house flies; Dimetilan in acetone applied topically to the notum.

Table 2.—The effect of two synergists upon mortality from Dimetilan by topical application at concentrations of 0.1 μg./fly for susceptible, 0.2 μg./fly for DDT-resistant, and 0.5 μg./fly for parathion-resistant strains.

Strain of House Fly	Per Cent Mortality After 24 Hours						
	Dimetilan	+Sesamex (μg.)			+Benzenesulfonate Ester (μg.)		
		0.02	0.05	0.1	0.02	0.05	0.1
Susceptible	48[a]	96	—	100	92	—	96
DDT-resistant	80	88	100	100	88	92	100
Parathion-resistant	64	—	92	96	—	100	100

[a] Numbers below double line are mortality percentages.

showed a low cross resistance (LD_{50} increased four-fold). Table 2 gives the data obtained from the application of the two synergists and Dimetilan on the three strains of flies.

The data for Isolan is given in figure 3 and table 3. Both the DDT- and parathion-resistant flies exhibited a low cross resistance to Isolan. (LD_{50} increased three- and four-fold, respectively.)

Figure 4 shows the ld-p lines for Pyrolan on the three strains. Pyrolan is the least effective of the three Geigy carbamates on susceptible flies. DDT- and parathion-resistant flies exhibited more cross resistance toward

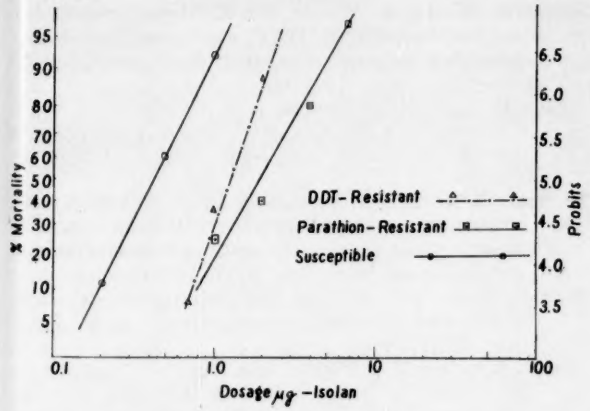


FIG. 3.—Probit-log dosage lines for three strains of house flies; Isolan in acetone applied topically to the notum.

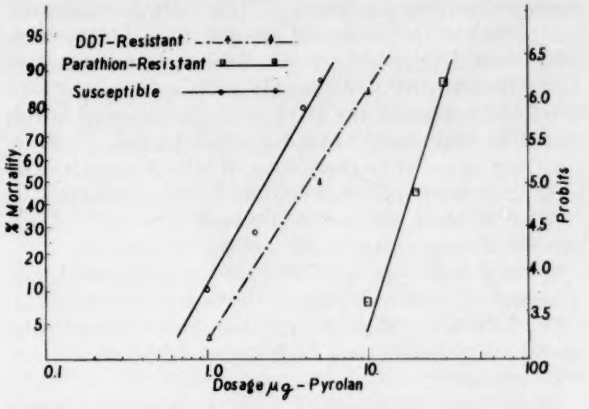


FIG. 4.—Probit-log dosage lines for three strains of house flies; Pyrolan in acetone applied topically to the notum.

Table 3.—The effect of two synergists upon mortality from Isolan by topical application at concentrations of 0.5 μg./fly for susceptible strains, and 1 μg./fly for DDT- and parathion-resistant strains.

Strain of House Fly	Per Cent Mortality After 24 Hours						
		+Sesamex (μg.)			+Benzenesulfonate Ester (μg.)		
	Isolan	0.02	0.05	0.1	0.02	0.05	0.1
Susceptible	44[a]	96	96	100	92	100	100
DDT-resistant	34	88	96	96	88	92	100
Parathion-resistant[b]	25	—	—	68[b]	—	—	92[b]

[a] Numbers below double line are mortality percentages.
[b] Using concentration of 0.5 μg./fly of the synergist gave 100% mortality for both synergists.

Table 4.—The effect of two synergists upon mortality from Pyrolan by topical application at a concentration of 2 μg./fly in three strains of house flies.

Strain of House Fly	Per Cent Mortality After 24 Hours						
		+Sesamex (μg.)			+Benzenesulfonate Ester (μg.)		
	Pyrolan	0.2	0.5	1.0	0.2	0.5	1.0
Susceptible	45[a]	—	92	100	—	92	100
DDT-resistant	25	92	84	100	92	88	100
Parathion-resistant	0.0	68	96	100	40	80	100

[a] Numbers below double line are mortality percentages.

Pyrolan than the previous two Geigy carbamates. Table 4 shows the effect of the synergists with Pyrolan. It is noted from the data in these four tables that both synergists do activate the four carbamates against the resistant as well as the susceptible flies.

In vitro inhibition determinations were carried out for the four carbamates on human blood plasma and house fly head cholinesterases. The molar concentrations at I_{50} values, mammalian toxicity to rats (oral application), and LD_{50} values of the three fly strains are listed in table 5. It is of interest to note that only Sevin is more active on house fly head cholinesterases while the other three carbamates show the reverse, being more active inhibitors on the human plasma cholinesterase. It is also noted that I_{50} values for the three Geigy carbamates are in reversed order to the LD_{50} values.

DISCUSSION.—The DDT- and parathion-resistant flies showed different levels of cross resistance to the four test carbamates. As an explanation of this phenomenon, we advance the following postulation: the detoxifying factor(s) for compounds having the structural formulas of the carbamate type are selected simultaneously during the selection for DDT or parathion resistance. This is especially possible if we suppose that the carbamate detoxifying factor(s) are linked to the DDT- and parathion-resistant factors. The fact that the resistant strains showed different levels of cross resistance to the four test compounds might be due to different rates of detoxification.

However, with the DDT-resistant strain of codling

Table 5.—Comparison between the four carbamates for toxicity to different strains of flies, rats, and the molar concentrations for *in vitro* I_{50} for human plasma and flyheads CHE.

Carbamates	LD_{50} Values (μg.)			P-R / DDT-R[a]	Molar Concentration at I_{50}		LD_{50} Rats[b] (Mg./Kg.)
	Susceptible	DDT-resistant	Parathion-resistant		Human Plasma	House Fly Heads	
Sevin	2.6	27	50	2	17.5×10^{-6}	3×10^{-6}	2000
Dimetilan	.11	.095	.37	3.9	8.2×10^{-7}	16.5×10^{-6}	35
Isolan	.42	1.3	2.0	1.5	3.0×10^{-7}	5.2×10^{-6}	54
Pyrolan	2.3	4.3	20	4.6	7.3×10^{-8}	6.6×10^{-7}	62

[a] $\frac{P-R}{DDT-R}$ = Ratio of LD_{50} Parathion-R/LD_{50} DDT-Resistant
[b] Information supplied by manufacturers.

moth (*Carpocapsa pomonella* (*L.*)), Sevin was found to be very effective for control Madsen & Hoyt (1958). This strain had developed only about four-fold resistance to DDT (Barnes 1958). When this result is compared with 100-fold increase for the DDT-resistant flies used in this study, we may suggest that the concentration of the detoxifying factor at an early stage of selection is not sufficient to produce any cross resistance effect to Sevin.

Work is being planned to determine the effect of the synergists on the detoxification of the carbamates.

CONCLUSIONS.—(1) DDT- and parathion-resistant flies showed cross resistance to the four test carbamates;

(2) Two 3,4-methylenedioxyphenyl derivatives synergize the carbamates to a high degree when used at low concentrations;

(3) With the exception of Sevin the other carbamates seem to be less active inhibitors on house fly head cholinesterases than on human plasma cholinesterases.

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Spotted Alfalfa Aphid Resistance in Mature Growth of Alfalfa Varieties[1]

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ABSTRACT

Mature stands of alfalfa varieties of domestic and foreign origin exhibited a wide range in suitability for population increase and plant injury by the spotted alfalfa aphid, *Therioaphis maculata* (Buck.). Varieties rated resistant—Lahontan, Moapa, Zia, Bam, and Sirsa No. 9—carried low populations and made excellent growth under conditions of epidemic infestation at high temperatures. Forty-three other varieties, classed intermediate and susceptible, supported heavy aphid populations that caused different degrees of plant injury. A wide range in tolerance was indicated by mortality rates of 2 to more than 90% for plants supporting 300 or more aphids per stem. Varieties of Turkistan parentage were generally tolerant to the aphid. The regrowth of infested varieties after cutback varied inversely with plant mortality.

Since the spotted alfalfa aphid, *Therioaphis maculata* (Buck.), was first discovered in New Mexico in 1954, a major portion of the alfalfa-growing area west of the Mississippi has been invaded. All recommended alfalfa varieties, with the probable exception of Lahontan, have occasionally suffered severe losses in yield, stand, and forage quality. Frequently, seedling stands have been killed in such a short time that replanting was necessary.

Appraisals of varietal differences in resistance to this aphid have generally been made on seedling alfalfa, considering plant mortality as the primary criterion of injury. The most extensive evaluations in the field have been made by Nielson *et al.* (1957) and in the greenhouse by Hackerott *et al.* (1958). Varieties found most resistant as seedlings were Lahontan, Nemastan, Moapa, and Zia (New Mexico 16), with mortalities ranging from about 20% to 50%. More than 90% of the susceptible entries were often killed. In contrast to the rather high seedling mortality in Lahontan, mature plants of this variety were seldom killed in heavy infestations observed in several nurseries. In the Moapa Valley of southern Nevada, Lahontan had little stand kill under population levels that injured and reduced yields in African, Caliverde, and California Common (Howe & Smith 1957). Mature growth of other varieties, particularly those of at least partial Turkistan origin (Orestan, Hardistan, and Ranger), tolerated heavy populations (Howe & Smith 1956).

It was evident that alfalfa varieties vary greatly in the injury they sustain under similar infestations and that mature growth response to the aphid may differ from that of seedlings. A study of the suitability of mature plants of several alfalfa varieties for aphid buildup and the damage under conditions favorable for rapid growth was conducted at Bakersfield, California, in 1957.

NURSERY PROCEDURES.—Two nursery tests were

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conducted. Entries in the first test were replicated four times and consisted of domestically marketed and certain experimental varieties. The second test included foreign, experimental, and check varieties in which seed quantities were insufficient for more than two replicates.

Seeds were hand-planted in sandy loam soil in single-row plots 10 feet long and 17 inches apart at the rate of 2.5 grams per row. Rows were placed in seven-row blocks between parallel check dams to facilitate irrigation. Each block contained a susceptible check, Caliverde, and a resistant check, Lahontan. An unplanted buffer strip 3 feet wide separated the blocks. All plantings were made between February 28 and March 4.

Satisfactory plant emergence was obtained with nearly all varieties. Overhead sprinkler irrigation was used until stands were established. No loss of stand was noticeable from any cause before severe aphid buildup. All varieties were growing vigorously and appeared healthy when the first spotted alfalfa aphids, mostly alate adults, were observed on April 3. Moderate ladybeetle populations that had developed on early pea aphid (*Macrosiphum pisi* (Harris)) infestations appeared to retard aphid buildup. One of three recently introduced parasites, *Praon palitans* Muesebeck, also retarded buildup during April and May, when as many as 12 to 15 mummies were observed on a single leaflet.

Several measures were taken to hasten the development of an injurious infestation. In mid-April, furrow irrigation was substituted for sprinkling. Before each irrigation furrows were reestablished to decrease washing of aphids from the stem bases. Counts made on April 16 revealed as many as 10 aphids per stem on susceptible varieties. Until mid-May, populations remained static without reaching levels sufficient for severe plant injury. In order to hasten aphid buildup, a 25% methoxychlor dusting schedule was set up to reduce predator populations. Selective activity by this insecticide was apparent, as aphid populations increased rapidly after treatments were begun, whereas untreated plots about 100 yards away carried low populations throughout the growing season. Finally, plants in the first test were cut back, 3 to 4 inches of stubble being left to assist in maintaining aphid populations.

APPRAISAL OF RESISTANCE.—The resistance of a variety was determined by appraising the combined effects of aphid population and plant injury. In the first test two aphid counts were made and plant injury was estimated on the basis of plant growth and mortality, whereas in the second only one aphid count was made and injury was based only on regrowth observations.

Infestation levels were determined from the total number of aphids per stem. On each observation date counts were made on five stems removed at random from each plot. High populations could be estimated most accurately during the cool of the morning when aphids were least active. Stems with 300 aphids or more were considered to represent plants with high host suitability. The injured stems from the most susceptible varieties supported at least this population, whereas the longer, healthier stems of certain tolerant varieties often supported much larger populations.

Plant injury, the second criterion of resistance, was appraised by determining average growth and mortality. Growth served as a measure of the plant's capacity to produce forage under severe aphid attack. It was evaluated on a scale of 1 to 10, a rating of 10 being assigned to plants with no visible regrowth. Later this rating was converted to the percentage of the maximum observed on uninjured Lahontan. Ratings were made 10 days after cutback.

Plant mortality rates were based on estimates of the linear portion of the row that was killed. The large num-

Table 1.—Classifications used for evaluating resistance in mature growth of alfalfa varieties to the spotted alfalfa aphid under conditions of epidemic infestation and rapid alfalfa growth. June–July 1957.

CLASSIFICATION	APPLIED POPULATION	PLANT INJURY	
		Per Cent of Maximum Growth During Attack	Mortality
Resistant			
High antibiosis	Reproduction effectively retarded. Limited feeding on most plants	93–100. Little stunting or honeydew	None to very little
Intermediate			
Group A. High tolerance, moderate antibiosis	Some reduction but sufficient aphids to cause severe honeydew	60–75. Most plants green	0–30%. Very slow
Group B. High tolerance, low antibiosis	Increase not retarded, thus about same as on living susceptible plants	45–65. Foliage tends to remain green but sticky	0–30%. Slow
Group C. Moderate tolerance, low antibiosis	Same as for B	33–58. Severe stunting, many plants with no growth	30–50%. Slower than in susceptible varieties
Susceptible			
Low tolerance, low antibiosis	Same as for intermediate group B except increase retarded by rapid killing of plants	18–38. Slight regrowth on some survivors	50–80%. Rapid killing
Highly susceptible			
Minimum tolerance and antibiosis	Same as for susceptible	13–20. Usually no regrowth	Over 80%. Very rapid killing

ber of plants made it impractical to count the living and dead plants, except in the few plots where stands were incomplete owing to poor seed germination. Plants were considered dead when no green was visible, for such plants seldom recovered.

RESULTS.—*Test No. 1.*—Aphid populations, plant growth, and plant mortality occurred in different patterns. Varieties were placed in various classifications, shown in figure 1, by interpreting the relationships between aphid populations and plant injury. The intermediate grouping was subdivided to identify varieties displaying some antibiosis and tolerance. The criteria used to classify varieties in relation to observations are outlined in table 1. Under the conditions of this test, low aphid populations were considered to be associated with antibiosis, since in concurrent studies in adjacent clonal nurseries low populations on stems were correlated closely with low reproduction and high mortality previously observed in antibiosis tests on the same plants. It is evident that in only a few varieties was antibiosis high enough to reduce aphid buildup. The low population levels on Lahontan and Bam caused no visible injury and no honeydew problem. It is probable that many of the winged aphids on these plants were migrants from nearby overpopulated injured susceptible varieties. Despite this, differences in population on adjacent resistant and susceptible plots were striking.

The plant mortality and plant growth columns in figure 1 show a wide range among alfalfa varieties in tolerance to the aphid. Differences in growth and injury in a portion of the planting are shown in figure 2. Tolerance to this aphid is especially important in areas where resistant varieties are not yet available.

In areas where an aphid buildup may occur after the last fall cutting, tolerance in varieties such as Ranger has undoubtedly prevented severe stand losses, since chemical control would seldom be used after harvest is complete. Most varieties in intermediate groups A and B can survive heavy infestations but suffer severely in forage production and quality. As in previous field observations (Howe & Smith 1956), heavily infested varieties of partial or total Turkistan origin (Ranger, Orestan, A-224, A-169, and A-600[3]) generally made more growth than other varieties. It is evident, as shown in figure 1, that the growth capacity of alfalfa under conditions of epidemic infestation varied inversely with plant mortality—low growth correlating with high mortality. This correlation concurs with field observations that heavily infested plants producing little or no regrowth seldom survived. Differences in ability to tolerate the toxic effects caused by the aphids, as described by Paschke & Sylvester (1957), probably account for the wide range in plant injury. Inherent plant vigor probably influenced survival slightly, though two varieties adapted to southern California, Caliverde and California Common 49, were among the more susceptible.

Test No. 2.—The following alfalfa varieties were observed in this test:

Domestic Varieties	*Foreign Varieties*
Resistant	
Lahontan	Sirsa No. 9 (India)
Moapa	
Zia	
Intermediate	
African	Lucerne Doehnfeldt (Denmark)
A-600	
Susceptible	
	Italian
Indian	Molecana
Hilmar	San Pastoni
Hairy Peruvian	Sweden
Caliverde	Umbra
Socheville	Ascalonia
Sevelra	
	Mexican
	Valenciana
	Tanhuato
	Apaseo
	Wahara
	Other
	Uruguayan clone 10
	Hungarian

Populations on all susceptible varieties were substantially more than 300 aphids per stem before cutting. Injury to these varieties was severe and little recovery was made after cutting; therefore, aphid populations fell below 300 per stem, owing to insufficient regrowth. It is evident that population level and degree of injury on mature alfalfa of susceptible varieties at the time of cutting affected patterns of injury and regrowth. The resistant varieties in this test carried populations ranging from 4 to 12 aphids per stem and produced uninjured honeydew-free growth. African and A-600 had aphid populations and injury similar to that observed in test 1. Six domestic and 11 foreign varieties were susceptible with few living plants appearing at the conclusion of the test. Sirsa No. 9 was the only foreign variety showing resistance in this test.

DISCUSSION.—Under the warm weather conditions of this experiment, only those varieties classed as resistant were sufficiently free of aphids and plant injury to provide economic control. All other varieties showed damage caused by stunting, honeydew contamination, and plant mortality.

Plant mortality among most varieties was considerably lower than that observed in seedlings by Hackerott *et al.* (1958) and Nielsen *et al.* (1957). This difference is attributed to increased tolerance in mature plant growth. The most resistant varieties in these tests were also the most resistant in the seedling studies. All varieties susceptible as mature stands were also susceptible as seedlings. The greatest difference in reaction between mature growth and seedlings was in certain intermediate varieties—such as Ladak, Cossack, and Rhizoma—which usually appeared much more tolerant as mature plants. Previous research (Howe & Smith 1957) indicates that certain resistant plants that would not support aphids at high temperatures would harbor small reproducing colonies at cooler temperatures in the fall. It is therefore probable that the more resistant varieties studied here could support higher populations with increased honeydew during cooler weather. Undoubtedly, other environmental conditions would also cause differences in resistance. The overall ranking of alfalfa varieties here generally corre-

[3] A-600 to be released as Culver by the Purdue Agricultural Experiment Station.

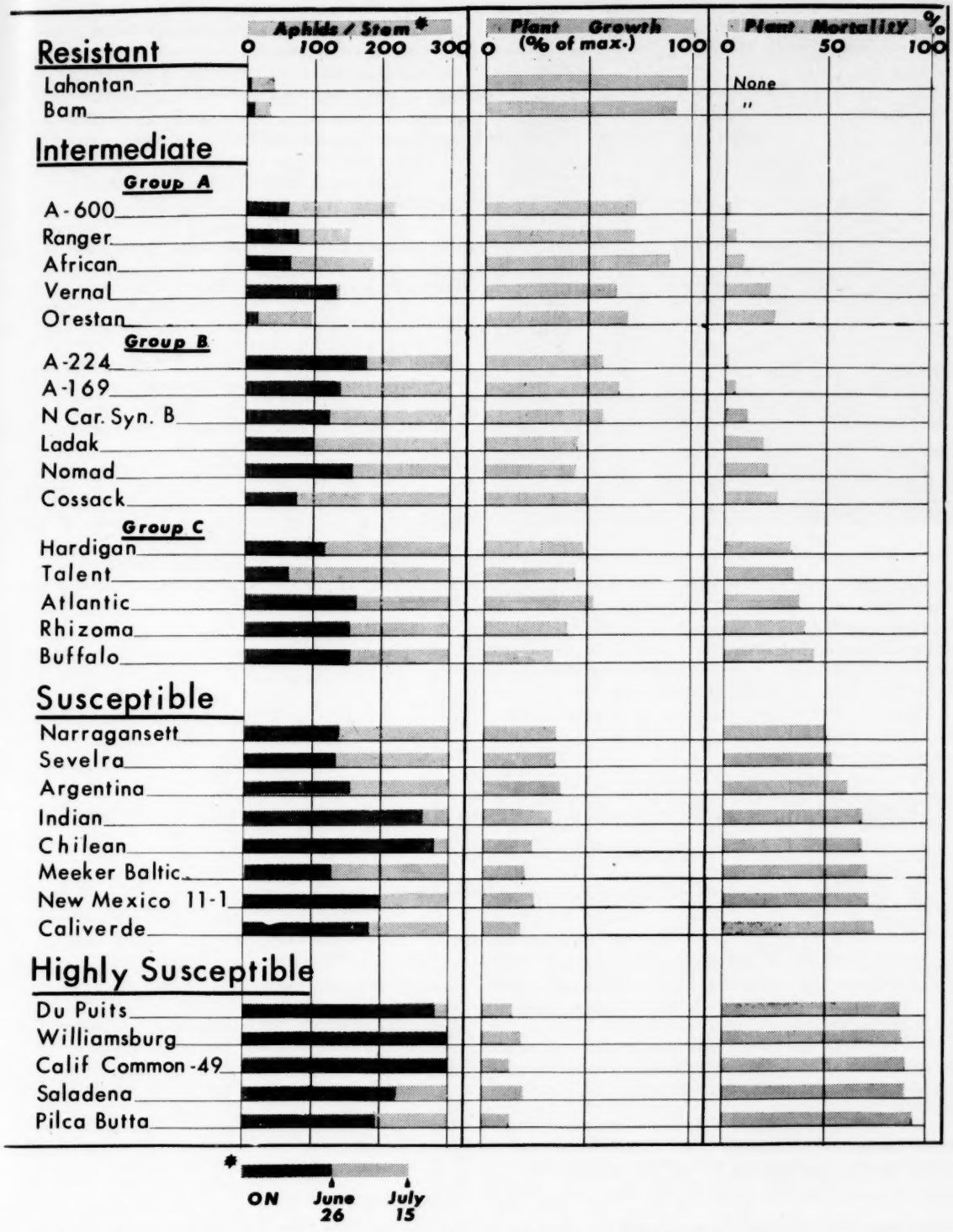

FIG. 1.—Spotted alfalfa aphid population levels, plant growth, and plant mortality in several alfalfa varieties when subjected to nursery infestations in the mature stage of growth in dry, clear weather with daytime temperature highs of 95° to 105°F.

FIG. 2.—Portions of alfalfa nursery plots showing differences in growth and injury to established stands of varieties when heavily infested with spotted alfalfa aphids. Plot 1: (a) Lahontan, (b) Bam, (c) A-600, (d) Caliverde, and (e) African. Plot 2: (a) Pilca Butta, (b) Chilean, (c) Saladena, (d) Italian, (e) Orestan, (f) Caliverde, and (g) Lahontan. Plot 3: (a) DuPuits, (b) Lucerne Doehnfeldt, (c) Caliverde, (d) Sevelra, (e) Saladena, (f) Bam, and (g) Lahontan.

lates with numerous field and nursery observations made during epidemics since 1955. The development of aphid biotypes that can survive and reproduce on resistant plants, as described by Stanford & McMurtry (1959) and Pesho *et al.* (1960), may also affect the expression of resistance in alfalfa to the spotted alfalfa aphid in the future. Thus far, alfalfa resistance to this aphid has demonstrated considerable stability at different localities and under different weather conditions.

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Effectiveness of Mixtures of Acrylonitrile and Carbon Tetrachloride against Three Pests of Stored Corn[1]

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ABSTRACT

Maize heavily infested with a complex of the rice weevil, *Sitophilus oryza* (L.) (-*Calandra oryzae* L.), *Sitotroga cerealella* (Oliv.), the Angoumois grain moth, and *Tribolium castaneum* (Hbst.) the red flour beetle, was fumigated with 1:2, 1:4, 1:6, and 1:8, by weight, mixtures of acrylonitrile:carbon tetrachloride at dosages of 0.05, 0.10, and 0.20 ml. of fumigant per 3 liters of grain. Similar maize was fumigated with a 3:1, by volume, mixture of ethylene dichloride:carbon tetrachloride at a dosage of 0.40 ml. per 3 liters of grain as a standard for comparison. The grain was fumigated for 24 hours. Counts were made of living adults of each species 24 hours and 6 weeks after the fumigants were applied. The two higher dosages of the 1:2 mixture and the highest dosage of the other mixtures were considered effective against all three species at the end of 24 hours. Only the highest dosage of the 1:2 mixture and the standard fumigant were considered satisfactory against the three species at the end of 6 weeks. Indirect evidence of competition between *S. oryza* and *S. cerealella* was found. As the low dosages and concentrations of acrylonitrile were not satisfactory, the use of such mixtures as general grain fumigants in Colombia does not appear practical.

The most common and damaging of the many pests that attack stored corn in the warmer regions of Colombia are the rice weevil, *Sitophilus oryza* (L.), the Angoumois grain moth, *Sitotroga cerealella* (Oliv.), and the red flour beetle, *Tribolium castaneum* (Hbst.). It has frequently been observed that these species make their appearance in freshly stored corn in clean bins in the following order: 1) a large number of the Angoumois grain moth; 2) a slow increase in the number of the rice weevil with a corre-

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sponding decrease in the number of Angoumois grain moth; 3) an increase in the number of red flour beetle with an increased number of either of the other species. The simultaneous reduction in the number of Angoumois grain moth and increase in the number of the rice weevil has been attributed to competition between these two species of the type described by Crombie (1944) as occurring between the Angoumois grain moth and the lesser grain borer, *Rhyzopertha dominica* (F.). The flour beetle can increase only slowly in sound grains (Daniels 1956); its increase with the increase of the other pests results from the broken grains and flour made by the other species.

Acrylonitrile is known to be more effective against both the rice weevil and the granary weevil, *Sitophilus granarius* (L.), than against the confused flour beetle, *Tribolium confusum* Duv. (Cotton & Young 1943, Lindgren & Vincent 1951, Lindgren *et al.* 1954), while carbon tetrachloride has been found to be more effective against *T. confusum* than against *S. granarius* (Lindgren & Vincent *op. cit.*). In preliminary tests (Restrepo, Saldarriaga, & Bravo, unpublished data) a 1:2, by weight, mixture of acrylonitrile:carbon tetrachloride proved more effective against adults of the rice weevil than against the adult red flour beetle; carbon tetrachloride alone proved more effective against the red flour beetle than against the rice weevil. In subsequent testing (Saldarriaga, Adarve, & Restrepo, unpublished data), low dosages of 1:5 and 1:8, by weight, mixtures of acrylonitrile:carbon tetrachloride gave nearly complete kills of the adults of both species after a 24-hour exposure to the fumigants. Such mixtures, by increasing the range of effectiveness and reducing the dosage of the highly toxic acrylonitrile, seemed to have promise for use in stored corn in Colombia. An experiment designed to test the effectiveness of these mixtures against the immature forms and adults of the Angoumois grain moth as well as all stages of the rice weevil and red flour beetle, was made at the government experiment station near Palmira, Valle del Cauca, Colombia, late in 1957. The results obtained are presented in this article.

METHODS.—Shelled corn of the hybrid "Diacol H-204," heavily infested with all stages of the three major pests, was thoroughly mixed to obtain the greatest possible uniformity. Heavily infested grain was used, since it has been found repeatedly that problems in commercial fumigations arise from the failure of the fumigants to give satisfactory control of heavily infested "spots" in the grain. Three samples of the corn were taken to determine the moisture content of the grain, the species and numbers of the pests per liter of grain, and the number of exit holes of the insects per 1,000 grains. The averages of these factors in the samples were: 14.35% moisture content; 14 adult rice weevils, 4 adult Angoumois grain moths, and 27 adult red flour beetles per liter of grain; and 446 exit holes per 1,000 grains. A few adults and larvae of the Indian-meal moth, *Plodia interpunctella* (Hbn.), and the cadelle, *Tenebroides mauritanicus* (L.), and adults of *Pagiocerus fiorii* (Egg.), *Zabrotes subfasciatus* (Boh.), and unidentified parasites were also noted in the grain.

Samples of 2 kg. of this corn were placed in cylindrical tin cans of approximately 3 liters capacity. Mixtures of acrylonitrile:carbon tetrachloride at the proportions, by weight, of 1:2, 1:4, 1:6, and 1:8, each at dosages of 0.05, 0.10, and 0.20 ml. per can, were placed in separate cans by means of a calibrated syringe. One can of grain was left untreated as a check and another can of grain was treated with 0.40 ml. of a 3:1, by volume, mixture of ethylene dichloride:carbon tetrachloride as a standard for comparison with the trial mixtures. The ethylene dichloride:carbon tetrachloride mixture was selected as the standard because, while slow in achieving a kill of the adults, it has proved satisfactory in the control of the species involved even in highly infested corn. Three replications were made of each treatment. The cans were sealed and placed in a randomized block arrangment in a storeroom immediately after the fumigant had been applied. To determine the effectiveness of the treatment against the adults of the pests, the cans were opened and a count was made of the living adults of each species found in each can 24 hours after the fumigants had been applied. The corn was then briefly aired, the living insects were replaced in the cans, and the cans were sealed and returned to the storeroom. A similar count was made 6 weeks (approximately one full generation) later in order to determine the effectiveness of the fumigants against the immature forms of the pests.

The statistical analysis of the data of this experiment was complicated by the fact that the species involved are interdependent and also by the fact that control of all three species was important. As the identification of the best experimental treatment and the comparison of the experimental treatments with the standard were primary objectives, a "damage index," a single number combining the effects of the three pests in each treatment, was adapted for analysis of the data. Fairfield-Smith (1936) presented a "selection index" for the analysis of data obtained in genetic studies. This index consists of a weighted sum of the factors studied, the weight assigned to each factor depending on the relative importance of that factor to the investigator and on its variability within treatments. Application of the Fairfield-Smith index to the data of the present experiment was straightforward. Weights assigned by the senior author to the different species corresponded to his estimates of their respective nuisance values: the rice weevil, 10; the Angoumois grain moth, 9; the red flour beetle, 2. The index was then determined from the data using the procedure outlined by Rao (1952, pp. 329–332). In making the analysis, the data were transformed to logarithms, since the range of variation of each species was roughly proportional to its mean (Bartlett 1947).

Although the damage index is useful for making statistical comparisons among treatments, it does not explain the reasons for the differences between "good" and "bad"

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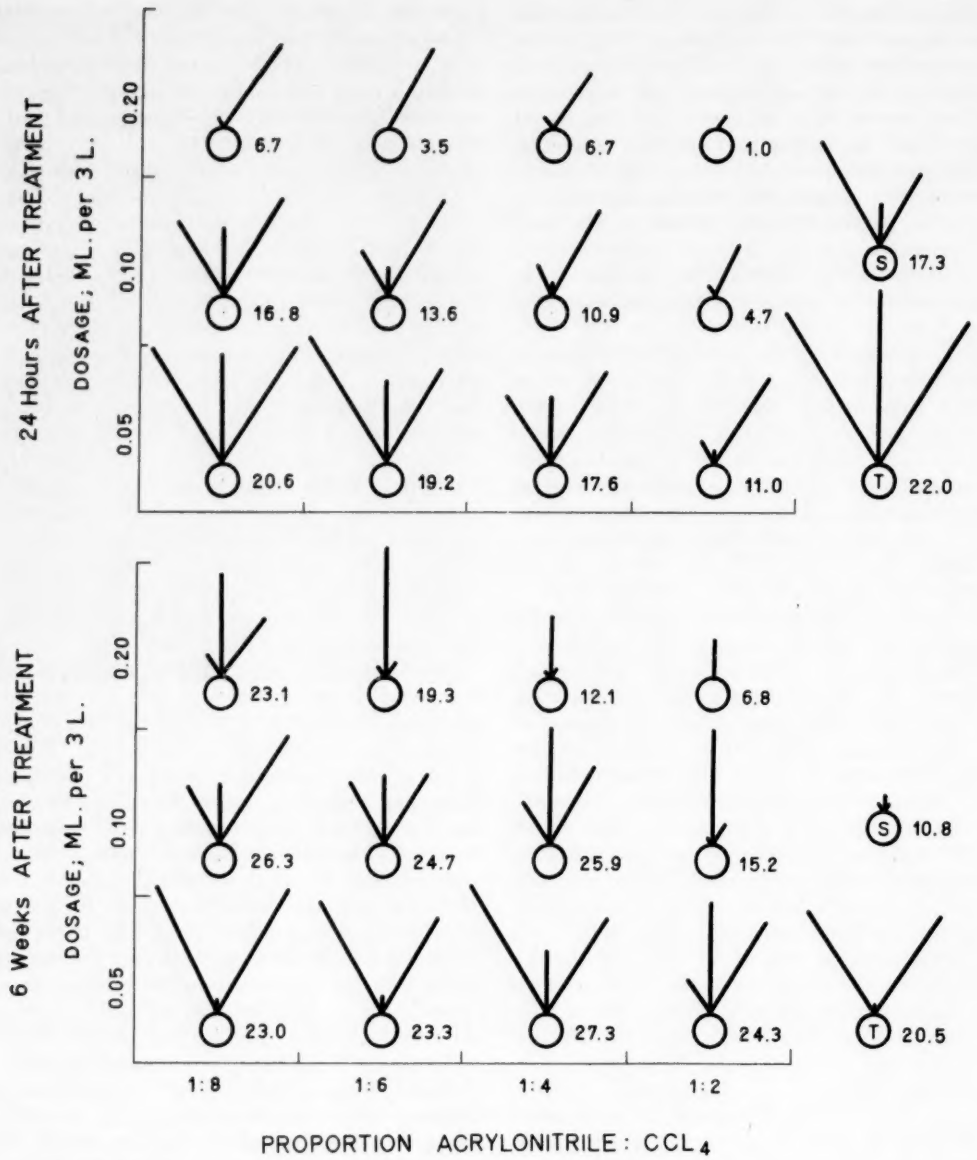

FIG. 1.—The selection index and the average number of three species of insects found in stored corn 24 hours (upper graph) and 6 weeks (lower graph) after fumigants had been applied. The glyphs marked "S" represent the standard treatment, those marked "T" the untreated check. The left rays of the glyphs represent the number of rice weevil, the center rays the number of Angoumois grain moth, and the right rays the number of red flour beetle. The number to the right of each glyph is the selection index for that treatment; the least significant differences at the 5% level for these indices are 4.3 for the data in the upper graph and 4.2 for the data in the lower graph.

treatments. When interactions between several factors are involved, such explanations are best visualized in graphic form. As the metroglyph method of Anderson (1957) proved most convenient for the data of the present experiment, metroglyphs were used to complement the numerical analysis.

RESULTS.—At the end of 24 hours, all the dosages of the 1:2 mixture, the two higher dosages of the 1:4 mixture, and the highest dosage of the other mixtures gave satisfactory mortalities of the rice weevil and Angoumois grain moth. The other treatments were not satisfactory in killing the adults of these pests. Only the two higher dosages of the 1:2 mixture produced an acceptable mortality of the red flour beetle at the end of the 24-hour period. When effectiveness against all three insects was considered, the two higher dosages of the 1:2 mixture and the highest dosage of the other mixtures gave acceptable control of the adults of these pests, while the other treatments failed to effect a rapid kill. The computed weights for this analysis were 9.4 for the rice weevil, 7.0 for the

Angoumois grain moth, and 2.0 for the red flour beetle.

At the end of 6 weeks, the two higher dosages of the 1:2 mixture, the highest dosage of the 1:4 and 1:6 mixtures, and the standard treatment gave excellent controls of the rice weevil and red flour beetle, while the other treatments were unsatisfactory against these pests. With the lowest dosage of the 1:4 and 1:8 mixtures there were slight increases over the untreated corn in the number of the rice weevil, but these increases were not significant. Similar increases in the number of the red flour beetle were obtained with the two lower dosages of the 1:8 mixture. Except for the lowest dosage of the 1:6 and 1:8 mixtures and the standard treatment, all of the treatments resulted in a significant increase in the numbers of the Angoumois grain moth over the untreated corn at the end of 6 weeks. The lowest and highest dosages of the 1:2 and 1:4 mixtures produced lesser numbers of the Angoumois grain moth than the medium dosages of these mixtures. There was also an increase in the number of the Angoumois grain moth with each increase in dosage of the 1:6 and 1:8 mixtures, and with each increase in concentration of acrylonitrile at the lowest dosage. When the effectiveness of the treatments against all species was considered, the standard treatment and the highest dosage of the 1:2 mixture were excellent and the other treatments were unsatisfactory at the end of the 6-week period. The computed weights for the analysis were 3.6 for the rice weevil, 3.4 for the Angoumois grain moth, and 2.0 for the red flour beetle. The results obtained in the two counts that were made in this experiment are presented in the figure. As the computed weights for the species were different in the two counts, the magnitudes of the selection indexes for both counts were made comparable by dividing each weight by the square root of the sum of all the weights in the index.

DISCUSSION.—The high effectiveness of acrylonitrile against the adults of the rice weevil and its rather poor action against the red flour beetle are in accord with the literature and previous tests. In this experiment, however, control of the red flour beetle did not increase with increased proportions of carbon tetrachloride, as was the case in the preliminary tests. This contradiction is probably due to the high population of the pest and the great amount of dockage in the grain used in the present experiment; Sun (1947) and Walkden & Schwitzgebel (1951) note the influence that these factors have on results obtained with fumigants. At the end of 24 hours, the standard treatment of ethylene dichloride:carbon tetrachloride was as effective against the adults of the red flour beetle as the mixtures of acrylonitrile:carbon tetrachloride, but less effective against the adults of the rice weevil and Angoumois grain moth. That its effectiveness against the red flour beetle was less in this experiment than in the other tests was probably caused by the factors noted above. While the mixtures of acrylonitrile with carbon tetrachloride were, in general, very good against the rice weevil, only the highest concentrations and dosages were satisfactory against the adults of all three species.

Six weeks after the fumigation marked changes in the effectiveness of the different materials were apparent. Changes in the effectiveness of fumigants against exposed adults and against hidden adults and immature forms are well known and can be caused by a variety of factors. In this specific case, the delayed action of ethylene dichloride (Richardson & Casanges 1942) and the poor penetration of acrylonitrile into grains (Kenaga, undated) undoubtedly combined with the specificity of these materials to produce the changes noted between the two counts. Only the standard treatment and the highest dosage of the 1:2 mixture were effective against the complex of the three species at the end of 6 weeks' time. The other mixtures were poor against the Angoumois grain moth and were satisfactory against the rice weevil and red flour beetle only at their highest dosage. At low dosages, therefore, none of the mixtures of acrylonitrile with carbon tetrachloride showed promise as grain fumigants for use in Colombia. The rapid effect of acrylonitrile against the adults of the pests and its specificity against the rice weevil, both demonstrated in this experiment, may be utilized in mixtures of this fumigant with more generally satisfactory materials.

The increased numbers of insects in some of the treated samples 6 weeks after the fumigants had been applied present some interesting points. A selective killing of the parasites noted in the cans prior to fumigation could have produced part of these increases. Unfortunately, the possible importance of the parasites was not known when the test was planned and no data were taken on the species or their numbers. The uniformly and radically increased number of the Angoumois grain moth with each increase in dosage of the lower concentrations of acrylonitrile indicates that some other factor was more dominant than the parasites. The metroglyphs clearly show a strong inverse relationship between the numbers of the rice weevil and the Angoumois grain moth. This relationship was so marked that the two species tended to cancel each other in the computation of the selection index, thus lowering the weights of their importance and raising the relative importance of the red flour beetle. The increase in the numbers of the Angoumois grain moth with decreasing numbers of the rice weevil strongly suggests competition between these two species. As the Angoumois grain moth was common in the corn at the time the fumigants were applied but nearly absent from the untreated plots 6 weeks later, the fumigant was probably applied at a critical period in the relationship between the species. A thorough study of the relationships between these two species is needed.

The selection index used in this experiment is a valid statistical procedure that could be utilized in analyzing multivariant data from many fields besides genetics, for which it was originally developed. There are, however, certain qualifications on its use. As the relative variabilities of the factors may change when the experiment on which the index is first computed is repeated, the weights for the different factors must be recalculated for each set of data. The need for this is well illustrated in the present experiment. Also, the index if considered by itself may obscure important results. For example, although the indices for the highest and lowest dosage of the 1:8 mixture were nearly equal 6 weeks after the fumigants were applied, there had been drastic changes in the relative numbers of the species on which these indices were based. The response of a single factor to the different treatments and the interactions between factors are

not shown in the selection index. Of the several graphic and tabular methods that were tried by the authors, the metroglyphic method proved to be the best, not only for rapid visualization of the data but also for suggesting hypotheses for statistical analysis and further experimentation. As noted by Anderson (*op. cit.*), the metroglyphic method should find a variety of uses in many fields.

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Effects of Nectariless Cottons on Populations of Three Lepidopterous Insects[1]

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ABSTRACT

In cage tests populations of the cotton leafworm (*Alabama argillacea* (Hbn.)) and the cabbage looper (*Trichoplusia ni* (Hbn.)) were seven to ten times as high on Empire, a cotton with extrafloral nectaries, as on nectariless cottons, but the presence of nectaries had little effect on the population of the pink bollworm (*Pectinophora gossypiella* (Saund.)).

The nectar from cotton plants has long been known as a source of food for many adult lepidopterous insects attacking cotton. Trelease (1879) reported that many species, particularly the bollworm (*Heliothis zea* (Boddie)) and cotton leafworm (*Alabama argillacea* (Hbn.)), came to the cotton plant to feed on the nectar secreted by the extrafloral nectaries.

Quaintance & Brues (1905) found that bollworm moths in the laboratory did not begin to oviposit until after they had been able to feed. They observed moths in the field seeking the nectar from extrafloral nectaries at the base of the flowers. They further noted, as did Fletcher (1929), that the bollworm was much more destructive to bottom-land cotton, where the soil was moist and fertile, than to the more stunted cotton in the uplands, and thought it probable that the moths were attracted to the ranker cotton because of its greater nectar supply.

Many insect populations do not become severe until cotton plants begin to bloom. Parsons & Marshall (1941), Anson *et al.* (1948), and MacDonald & Fielding (1938) found that the first appearance of flowers was a good indicator for the beginning of bollworm infestations on cotton, as egg laying did not begin until that time.

In a study of the oviposition sites of the pink bollworm moth (*Pectinophora gossypiella* (Saund.)), Brazzel & Martin (1957) noted an increase in the number of eggs laid on plants as the season advanced. Moths obtained from overwintering larvae were used in the experiment. Even though the authors did not mention food supply as a contributing factor, the increase in egg laying could be correlated with the beginning and increase of flowering of the plants. In the laboratory, pink bollworm moths laid about twice as many eggs when fed sugar solutions as when fed water only (Lukefahr & Griffin 1956).

Different species of cotton vary in the number of nectaries present. Commercial upland cotton, *Gossypium hirsutum* L., possesses both floral and extrafloral nectaries. One extrafloral nectary is found on the midrib of each true leaf, and sometimes on each of the two prominent veins attached to the midrib. Six involucral nectaries are located on the flower or boll, three at the base of the involucral bracts, and three between the calyx and involucre. The floral nectary is at the base of the inner side of the calyx. Floral nectaries are not considered of any value to the important lepidopterous insects attacking cotton, as the flowers are closed during their active period. According to Tyler (1908) and Reed (1917), *G. tomentosum* Nutt., a wild Hawaiian cotton, is the only naturally occurring species that is void of extrafloral nec-

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[2] In cooperation with the agricultural experiment stations of Texas and North Carolina.

taries. They also stated that the outer involucral nectaries were absent in all cultivated Asiatic cottons.

Experiments to determine the population increase of the cabbage looper (*Trichoplusia ni* (Hbn.)), the cotton leafworm, and the pink bollworm on cottons with and without nectaries were conducted at Brownsville, Texas, in 1957 and 1958.

TYPE OF COTTON USED.—Empire, a large-bolled, growth-determinate commercial variety of upland cotton with nectaries, was used for comparative purposes. The nectariless cottons were inbred generations from second and third backcross transferences of the nectariless character to upland cotton. Selection was practiced for the recessively inherited nectariless expression. The late-flowering thermophotoperiodic condition of the nectariless *tomentosum* parent was separated from the nectariless expression. The early-flowering behavior of upland cotton with a nectariless expression was considered desirable, but was incompletely attained. The cotton designated as Nectariless 1957 produced bolls that were less than half the size of commercial cotton. Nectaries were found on approximately 10% of the leaves, but many of them were not functional, and no nectaries were found on the bolls. Another cotton, designated as Nectariless 1958, showed only minor improvement in boll size, being half as large as Empire, and nectaries occurred on about 15% of the leaves and bolls. Meyer Nectariless is a completely nectariless strain, having been attained from *tomentosum* by hybridization.[3] It is early flowering and has bolls about one-fourth the size of commercial cotton.

PROCEDURE.—The nectariless and nectar-producing cottons were grown separately in screen cages 6×6×24 feet, and equal insect populations were introduced into cages containing equal numbers of plants. Flowers were present in all cages at the time the insects were introduced. All plants had been treated with demeton a week to 10 days before to prevent aphid buildup and thereby eliminate honeydew on which the moths could feed. About 3 weeks after the insects were introduced, population counts were made on the respective cottons.

The first of two experiments with the cabbage looper was conducted in 1957. Six cages were used, three planted with Empire and three with Nectariless 1957. Twelve pupae were placed in each cage on October 18. Counts were made of the larvae and pupae on four plants in each cage. In the other experiment with this insect in 1958, the same numbers of cages were installed on June 26, and four pairs of moths were released in each cage. Empire cotton was used again in three cages, Meyer Nectariless in two cages, and Nectariless 1957 in one cage. Counts were made of all insects on one of two rows of plants in each cage.

The first experiment with the cotton leafworm was begun on August 18, 1958. Four cages were used, two each with Empire and Nectariless 1958. Four pairs of moths were introduced into each cage. Counts were made of the pupae on 10 plants in each cage after almost all the larvae had pupated. A second experiment was begun on September 14 on cotton in the second cabbage looper experiment that had been cut back and allowed to grow until fruiting commenced. Eight pairs of leafworm moths were introduced into each cage. Counts were made of all the pupae on one row of plants in each cage.

Table 1.—Cotton leafworm and cabbage looper populations developing on Empire and nectariless cottons.

EXPERIMENT AND REPLICATE	NUMBER OF PUPAE AND/OR LARVAE	
	Empire	Nectariless
	Cabbage looper	
First (1957)		
1	20	3
2	28	0
3	11	4
Second (1958)		
1	32	4
2	100	8
3	106	10
	Cotton leafworm	
First (1958)		
1	107	23
2	129	29
Second (1958)		
1	112	0
2	281	27
3	306	41

In an experiment with the pink bollworm, plants in three cages each of Empire and Nectariless 1958 were infested with 50 eggs per cage on June 8, 1958. On June 27 three pairs of moths were liberated in each cage, and at weekly intervals until August 29 boll samples were taken for infestation counts.

RESULTS AND DISCUSSION.—The infestations of cabbage loopers and leafworms on all nectariless cottons were much lower than those on Empire (table 1). However, this difference between nectariless and Empire cottons was not so great in the first cotton leafworm experiment, using Nectariless 1958, as in the other experiments with both insects on Nectariless 1957 and Meyer Nectariless. The fact that Nectariless 1958 had nectaries on 15% of the bolls may have accounted for its higher infestation. The Empire cotton was usually defoliated by the insects during the experiment, but the nectariless cottons were only slightly damaged.

Shortly after the first leafworm experiment was begun, a heavy mealy bug infestation (undetermined species) developed in one of the nectariless cotton cages. This infestation was not controlled, and the leaves soon became sticky with honeydew. A heavy leafworm infestation developed on both types—186 pupae on the nectariless and 208 on the Empire cotton. This indicated that the moths fed on the honeydew when nectar was not available and that honeydew was as good a food as nectar. No data for this replicate are given in table 1.

A few plants of the various nectariless cottons grown beside a field of Empire did not show any larval preference between varieties. This observation indicated that it was the lack of food for the adults that reduced the number of eggs laid, thus preventing damaging infestations.

The pink bollworm tests showed very little differences between cottons. Both types of cotton had about the same boll infestation (46% and 48%); however, the average numbers of mines and larvae per boll were less on the

[3] Original backcross material supplied by James Meyer, Delta Branch Experiment Station, Stoneville, Miss.

nectariless cottons—1.6 and 0.7 as compared with 2.5 and 1.1 on Empire. It was rather difficult to compare these two cottons because of the smaller bolls and the presence of a few functional nectaries on the nectariless cotton. Cottons that are completely nectariless and with bolls comparable to commercial cottons may give considerable reduction in pink bollworm populations.

What effect the nectariless cottons will have under field conditions where there is free movement of insect populations remains to be seen. It is possible that strong-flying moths will feed on wild flowering plants and return to the cotton field for oviposition. On the other hand, the moths may not find nectariless cotton so attractive and will begin oviposition on other plants. However, during midsummer at Brownsville very few flowering plants other than irrigated cotton were available.

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Effect of Culm Solidness on the Survival of the Wheat Stem Sawfly[1]

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ABSTRACT

The use of wheat culm solidness as a measure of resistance to the wheat stem sawfly, *Cephus cinctus* Nort., has repeatedly been questioned. This study critically evaluated the solidness-resistance relationship of six wheat selections, which by commonly used measuring techniques were similar in solidness but varied in their degrees of resistance. Using sawfly survival as the criterion for resistance, a definite relationship between culm solidness and sawfly resistance was found to exist. Consistently significant differences in culm solidness of the test selections were found, but were most apparent when cross-section solidness readings through the pith cavities in the tops of the internodes were included with readings from the centers of the internodes. Fertilization had very little influence on the culm solidness relationships of the selections studied. From the data it appears that with adequate sampling even minor differences in culm solidness can be shown to exert significant influence on sawfly resistance.

Attempts to locate selections of wheat resistant to the wheat stem sawfly, *Cephus cinctus* Nort., were first made by Kemp (1934). He found that solid-culmed lines had less sawfly cutting than hollow-culmed lines. Farstad (1940) stated, "There is a direct relationship between the amount and consistency of the pith tissue present and the degree of effect upon the developing *C. cinctus*." Platt & Farstad (1941) as reported by Painter (1951) indicated, that "Whenever pith tissue completely fills the lumen of a stem, there appears to be a mechanical impediment to the movement of the larva. Larvae which hatch from eggs completely surrounded by pith do not survive." In further studies Platt & Farstad (1946) concluded that hollow-culmed varieties were generally susceptible and solid-culmed varieties generally resistant. But they also found differences in degree of susceptibility among hollow-culmed varieties, indicating that factors other than solidness might be involved in resistance.

Roberts (1954) studied types of resistance in wheat and found significant differences in sawfly reaction in: (1) infestability, (2) survival of the eggs and larvae from time of laying up to the feeding stage, and (3) survival of the feeding larvae up to the time of cutting. He found some association between stem solidness and the mortality of the eggs and early larvae. However, he stated, "When data on the survival of the older larvae are compared with data on stem solidness, little justification can be found for attempting to associate these two phenomena." He indicated that stem solidness or some result of stem solidness may be a factor in resistance, but that other important factors also are involved.

Luginbill & McNeal (1954) evaluated the effect of various fertilizers on sawfly cutting. Phosphorous applied alone or mixed with nitrogen at planting time resulted in increased sawfly cutting. They attributed this increase in cutting to physiological or structural changes in the lines tested which made them more susceptible.

Cereal grains such as barley and oats are also susceptible to infestation by the wheat stem sawfly. Barley

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is variable in its resistance, but in general only a small per cent of the sawflies survive. Oats may be infested, but the larvae die soon after hatching from the eggs. Both of these grains have hollow stems, indicating that factors other than solid culms act to prevent their successful existence in infested grasses.

Plant breeders and entomologists have examined many thousands of wheat selections in attempts to locate resistance to the wheat stem sawfly, either with solid culm characteristics or types of resistance similar to that demonstrated by oats or barley. Invariably, resistant selections were found to possess solid culms. Using the generally accepted procedure for evaluating solidness, these resistant selections have varied in both degree of solidness and degree of resistance. However, significant correlation between degree of solidness and degree of resistance was not always apparent. In order to evaluate more critically the relationship of solidness to resistance, this investigation was initiated.

PROCEDURE.—The investigation was concerned primarily with a study of antibiosis. Tolerance was not considered, and preference was determined to be of little consequence. Since solidness is variable with time and location owing to genetic and environmental effects, the only suitable measure of antibiosis is the determination of relative sawfly survival in the field. Six hard red spring wheat selections with similar solidness ratings but different levels of resistance were chosen for study. These were: N.D. 51-3355, Mont. B50-18, Mont. B51-16, Canada 4352-96, Canada 4336-125 and Rescue. The selections were observed for culm solidness in six experiments and sawfly infestation and survival in eight experiments conducted at four locations in North Dakota. Each experiment was replicated four times in a split-plot arrangement. Two fertility levels, (1) no fertilizer, and (2) 30 pounds of nitrogen plus 100 pounds of phosphorous (P_2O_5), were used. Individual plots consisted of two 10-foot rows spaced 1 foot apart. Analysis of variance techniques were applied to data from individual experiments as well as to combined data. A multiple range test outlined by Duncan (1955) was applied to aid in evaluating differences among means.

The sawfly infestation for each selection was determined in all experiments. Ten stems selected at random from each plot were split longitudinally from the base to the spikelet. If an egg, an egg scar, or a tunneling larva was encountered, the stem was considered infested. A percentage was recorded for the plot. These percentage figures were transformed by the arc sine transformation for proportions for use in the analyses of variance.

Culm solidness ratings were determined on an individual plot basis. Solidness readings were recorded from the main stem and a tiller from each of five plants randomly selected. Each culm was cross sectioned at the top and center of every above-ground internode and an estimate made of the solidness at the point of the cross section. When the culm appeared to be completely filled with pith, a reading of five was recorded. When the culm appeared to be completely hollow, a reading of one was recorded. Variation in the amount of pith tissue between readings of one and five were recorded as 2, 3, or 4, depending on the amount of pith tissue present. All readings on an individual culm were averaged to give a culm rating. The main stem and tiller ratings for a plot were then averaged to give a plot rating for solidness. These were used in the analyses of variance.

Table 1.—Combined solidness rating means of culm solidness with Duncan's 5% range test applied.

SELECTION	MEAN[a]
B50-18	3.96]
4336-125	3.99]
Rescue	4.06]
51-3355	4.15]
B51-16	4.17]
4352-96	4.26]

[a] Means falling in a bracket are similar.

Sawfly survival was used as an index of resistance in this study. The base sample consisted of 50 consecutive standing culms and sawfly-cut stubs. Two samples were taken from each plot, 1 foot in from either end of the second row of plants. The percentage of sawfly-cut stubs in a base sample was calculated. These figures were used in the analyses of variance after they were transformed by the arc sine transformation for proportions.

RESULTS.—Preference exhibited by the wheat stem sawfly was of little consequence in this study. Infestation varied from 60% to 97% with a mean infestation of 83% for all experiments. In only two of the the eight experiments were fertilized blocks significantly more infested than nonfertilized blocks. Measurable differences among selections occurred in three experiments. In every case a different selection caused the measurable variation. Consequently, these differences were not considered important in evaluating resistance in this investigation.

Consistently significant differences in culm solidness were found among selections. The selection 4352–96 had a significantly higher solidness rating than all other selections studied (table 1). In the statistical analysis of combined data from six experiments, two selections, B50-18 and 4336-125, had significantly lower solidness ratings than all others under investigation. Trends in individual experiment analyses indicated that B50-18 was significantly less solid than 4336-125. Differences were observed among fertilization treatments in only one experiment.

A comparison was made between a top and center cross-section reading and a top reading alone to determine what effect these readings had on the internode solidness rating. Solidness ratings were markedly decreased by using only the top cross-section readings, some lines being more adversely affected than others. This indicates that the center of a particular internode contains more pith than the area just below the node, and that the latter area varies more among selections than the center of the internode.

Sawfly survival was utilized as the index of measurable resistance found among selections. The data are summarized in table 2. Significant differences among selections occurred in all experiments. Selection 4352–96 was significantly more resistant and selection B50-18 was significantly less resistant than all other selections investigated. No differences of importance were found among the four other selections. Fertilization had very little effect, significant differences in survival being measured

Table 2.—Combined means of per cent sawfly survival with Duncan's 5% range test applied.

SELECTION	MEAN[a]
4352-96	16.5
51-3355	34.6
Rescue	34.7
B51-16	36.2
4336-125	37.4
B50-18	58.8

[a] Means falling in a bracket are similar.

in only one of the eight experiments.

Culm solidness and survival data indicated a strong negative relationship between these two characteristics. This association was studied by use of covariance analysis. Correlation coefficients, calculated on the basis of selection means, are presented in table 3.

DISCUSSION.—Culm solidness is associated with resistance. Significant differences were observed in sawfly survival and culm solidness among the six selections investigated. One selection, 4352-96, exhibited more resistance and a higher mean culm solidness rating than other selections. Another selection, B50-18, exhibited less resistance and a lower culm solidness rating than the other selections. Negative correlation coefficients strongly illustrated the inverse association with respect to culm solidness and sawfly survival.

An important reservation of the above statement exists. A culm solidness rating for a particular selection must be based upon a large number of observations before it can be determined with confidence. This fact is illustrated by the significant differences in selection means for culm solidness even though these differences are very small (example: 3.99 versus 4.06). This result would clarify the wheat breeders' difficulty in using solidness as a criterion for resistance. In early generations only one or a few plants can be evaluated. When selecting on the basis of solidness, the number of measurements is necessarily limited, and consequently the association between solidness and resistance cannot be adequately observed.

The cross-section reading from the top of the internode is important in determining the total solidness of a culm. This cross-section reading reveals a pith cavity common to the solid wheats. When it is included with a reading from the center of the internode it reduces the solidness rating and provides greater variability among selections. This fact is demonstrated by a comparison of 4352-96 and B50-18. The selection 4352-96 had one of the smallest pith cavity readings while B50-18 had the largest. Mean solidness ratings were reduced 0.10 and 0.24, respectively, when the cross sections from the tops of the internodes were used alone. The effect that this condition has on sawfly activity is unknown. The possibility exists that an egg that is deposited in this cavity may have a better chance for survival and development than one placed in pith tissue and surrounded by parenchyma. Lignification of the parenchyma cells around the egg occurs rapidly after the egg is laid. This lignification may inhibit egg development or movement of the small larva from the original site. This hypothesis would agree with Roberts (1954), who found association between culm solidness and the mortality of eggs and early larvae. Very little response from fertilization was observed in this investigation.

Table 3.—Associations between culm solidness and sawfly survival as measured by correlation coefficients.

EXPERIMENT	CORRELATION COEFFICIENTS OF SELECTIONS
1	−0.846*
2	−0.659
3	−0.822*
4	−0.930**
5	−0.606
6	−0.608

* At 5% level.
** At 1% level.

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Exposure of House Flies and Oriental Rat Fleas on a High-Altitude Balloon Flight[1]

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Abstract

House flies (*Musca domestica* L.) and oriental rat fleas (*Xenopsylla cheopis* (Rothsch.)) were exposed inside an air-conditioned gondola during a balloon flight that maintained an altitude of 78,000 to 82,000 feet for 16 hours. The purpose was to gather information on biological damage from cosmic and other radiations near the top of the atmosphere during a well-instrumented flight. Succeeding generations of the exposed insects reproduced about normally and no physical abnormalities were noted. Fleas exposed outside the gondola were killed by the low temperature.

The rapid approach of the day when man will have the means for travel in space necessitates gathering information on biological damage from cosmic and other high-altitude radiations. An easy way to obtain some of this information is to expose insects in balloons at altitudes of 80,000 to 100,000 feet. Ney & Winckler (1958) state that approximately one primary cosmic ray passes through each square centimeter at the top of the atmosphere per second at high latitudes. This is 60 times the intensity at sea level. Cosmic-ray intensity increases only slightly above 100,000 feet.

On July 26 and 27, 1958, several species of insects were exposed within or on the outside of a pressurized gondola of manned balloon fllight No. 793, Office of Naval Research Project Stratolab III, Winzen Research Inc. The results of a thorough search for genetic changes in *Drosophila melanogaster* Meig. exposed during this flight and a review of the effects on tissues of natural ionizing radiation, particularly the harmful heavy primary component of cosmic radiation, have been reported (Pipkin & Sullivan 1959). The present paper gives the results obtained by exposing house flies (*Musca domestica* L.) and oriental rat fleas (*Xenopsylla cheopis* (Rothsch.)) during the same flight.

Materials and Methods.—The house flies and oriental rat fleas were reared at the Orlando, Florida, laboratory of the Entomology Research Division. On July 16 or 17, 24 plastic containers with the insects in various stages of development were shipped by air mail, special delivery, to a location near the test site. Shipments were planned by telephone, and the insects arrived in good condition within 24 hours. House fly eggs and larvae were shipped in larval medium, and the pupae and adults were packed in loose excelsior. On the ascension date all flies had hatched and those shipped as pupae were adults. The fleas were sent as pupae and adults, also packed in loose excelsior. Each container held about 100 flies or 1000 to 5000 fleas.

The test vehicle was a 2×10^6 cubic foot ballon of 2-mil polyethylene with a pressurized gondola 7 feet in diameter attached. The house flies and fleas were placed inside the gondola in a kraft paper bag just before launching time. Vials containing fleas were also secured near the top on the outside of the gondola with No. 890 tape. The balloon, which was manned by Commander Malcolm Ross and Lee Lewis, was launched from an open-pit iron mine near Crosby, Minnesota (55° N.). The flight profile during the 34-hour exposure period and the temperatures inside and outside the gondola during this time are shown in fig. 1.

Table 1.—Results of tests with house flies exposed at 78,000 to 82,000 feet for 16 hours in a gondola during a balloon ascent.[a]

Stage When Shipped	Container No.	Mortality (%)	F_1 Emergence		F_2 Emergence	
			Males	Females	Males	Females
Eggs or larvae	Exposed					
	1	37	—	0	—	—
	2	49	0	0	—	—
	3	40	94	142	1050	1000
	Unexposed					
	10	4	143	142	603	430
	11	7	130	135	963	807
	12	3	0	0	—	—
	13	4	6	6	140	181
	15	2	10	25	940	535
	17	2	0	0	—	—
Pupae	Exposed					
	4	95	3	33	79	94
	5	85	9	54	800	485
	6	97	11	11	0	0
	7	100	—	—	—	—
	8	100	—	—	—	—
	9	100	—	—	—	—
	Unexposed					
	14	73	40	53	140	128
	16	92	6	5	76	66
	18	86	100	108	1150	750

[a] All adults of both F_1 and F_2 generations were normal.

Herman Yagoda, of the Air Force Cambridge Research Center, monitored the outside of the gondola with a small block of Ilford nuclear track emulsions. He stated, "The measured (heavy nucleus) thindown intensity is 1.06 ± 0.31 per cc. of emulsion per day of flight time above 60,000 feet, the thindown tracks tallied in this survey being of nuclear charge 6 (carbon) or higher." This value is in good agreement with earlier measurements in the Minnesota area at similar elevations (Yagoda 1956).

The personnel dosimetry data from the flight were evaluated by the Naval Medical Research Institute. The quartz-fiber pocket ionization chamber readings ranged from 14 to 20 milliroentgens of integrated dose for total time aloft. All film gamma-ray readings were below 20 milliroentgens.

When the insects were returned to Orlando on July 29, the flies were reared through two generations and observed for any deviation from the norm in appearance of growth.

Results.—The results with house flies are given in table 1. Those exposed within the gondola showed higher mortality than the unexposed checks. All the fleas exposed outside the gondola were killed by the extreme

[1] Accepted for publication October 2, 1959.

[2] The U. S. Office of Naval Research and Winzen Research, Inc., cooperated in these tests. G. C. Labrecque, H. G. Wilson, and Paul N. Mason of the Orlando, Florida, laboratory reared the insects and made the post-treatment observations.

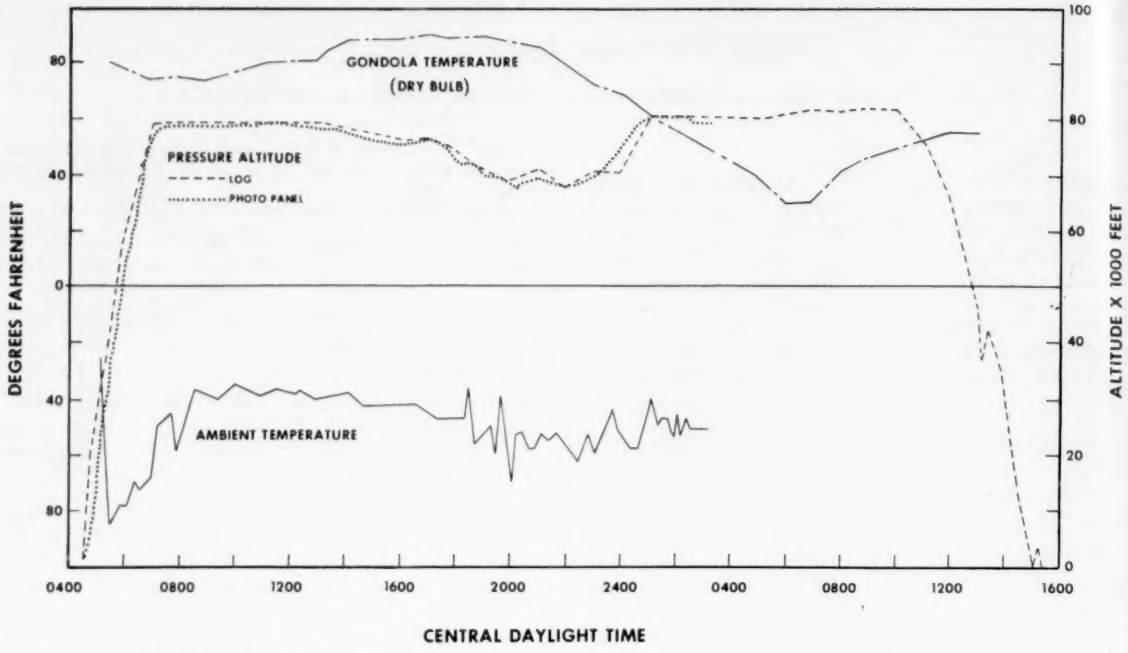

FIG. 1.—Flight profile during exposure of insects to high-altitude radiation, July 26–29, 1958.

cold at such high altitudes. In two containers exposed in the gondola less than half the fleas were killed, as compared with slightly more than half in three unexposed checks.

No genetic effects were apparent in either species. Reproduction in the succeeding generations was normal, and no physical abnormalities were noted.

These results are in agreement with the findings with *Drosophila* exposed during the same fllight. More evidence is needed, but it is reassuring that no genetic damage was observed from cosmic radiation when approximately 16,000 insects (including *Drosophila*) were exposed at such a high altitude for 16 hours.

It is planned to repeat work of this nature when means are available for exposing insects on recoverable satellites that orbit in the Great Radiation Belt, which reaches the first peak intensity at an altitude of about 2400 miles and peaks again at 10,000 miles. In each band bombardment of electrons at peak is equivalent to about 10 roentgens per hour and that of protons about 100 roentgens per hour.

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White-Pine Shoot Borer (*Eucosma gloriola* Heinrich)[1]

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ABSTRACT

The white-pine shoot borer (*Eucosma gloriola* Heinrich) was described from Connecticut collections on white pine in 1930. It was collected only a year later in Quebec and misidentified as Nantucket pine moth (*Rhyacionia frustrana* (Comst.)). This confusion has been common wherever the damage or immature insect was observed on hard pines.

Eucosma gloriola larvae were found boring in the pith of Scotch pine terminals at Knauers, Pennsylvania, in 1957, 1958, and 1959. Other conifers also harbored this pest. Its hosts, distribution, description of damage, fragmentary notes on the life history, and mention of a new species of parasite have been brought together to hasten the recognition and eventually the control of this important pest of pines.

The white-pine shoot borer (*Eucosma gloriola* Heinr.) has been reported as a pest of white pine (*Pinus strobus*

[1] Accepted for publication October 7, 1959.

[2] Drs. J. W. Butcher, Assistant Professor of Entomology, Michigan State University; T. S. DeLong, Head Nurseryman, Pennsylvania Department of Forests and Waters, and W. E. Waters, Entomologist, Northeastern Forest Experiment Station, Forest Service, New Haven, Connecticut, kindly reviewed the manuscript of this paper. Their suggestions are appreciated.

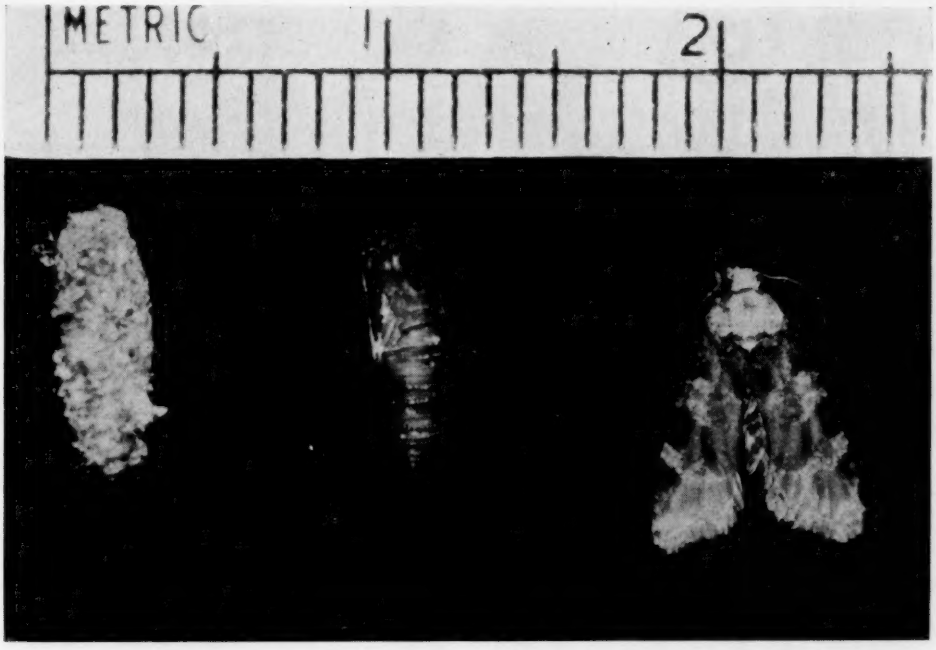

FIG. 1.—L. to R. Thin silken cocoon covered with soil particles, pupal cast skin, and moth of *Eucosma gloriola*.

L.) since 1930, when it was first observed in Connecticut (Craighead 1950). It is almost certain that this moth was widespread in northeastern United States and at least in Quebec, Canada, at this time. The unfortunate confusion in distinguishing its damage from that of the Nantucket pine moth (*Rhyacionia frustrana* (Comst.)) has impeded recognition of the importance of the *Eucosma*. The common name—white-pine shoot borer—probably also has misled many persons into discounting it as an insect pest of hard pines or other conifers. Indeed the Nantucket pine moth was reported causing damage to Scotch and red pine (*Pinus sylvestris* L. and *P. resinosa* Ait.) at Berthierville, Quebec, in 1931; but, in fact, the insect responsible was the white-pine shoot borer (Daviault 1932, 1942). The situation was similar in Ontario (Brown 1942).

South of the Canadian border there has been only one published reference in which a conifer other than white pine was listed as a host. Shenefelt & Benjamin (1955) listed white and red pines as host species.

Such was the status of information when a request came in June 1957 from Thomas S. DeLong, of Knauers, Pennsylvania, a community south of Reading, to determine the cause of terminal injury to Scotch pine in his Christmas tree planting. Damage to the trees resembled that reported caused by *Eucosma sonomana* (Kearf.) to jack pine (*Pinus banksiana* Lamb) by Butcher & Hodson (1949). An unsuccessful attempt was made to rear larvae to the adult stage for specific determination in 1957. Force-rearing in the manner of Butcher & Hodson (1949) of stock collected in June 1958 produced moths in 5 to 6 months. Mr. H. W. Capps, of the U. S. Department of Agriculture, Entomology Research Division, kindly determined the moths as belonging to the species, *Eucosma gloriola* Heinrich (fig. 1).

HOSTS.—White pine and Scotch pine are favored equally in the plantation at Knauers. The insect has been observed there also feeding in a very few red pine, Austrian pine (*Pinus nigra* Arnold), and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). Pitch pine (*P. rigida* Mill.) near Pine Grove Furnace, Pennsylvania, showed evidence of attack by the same insect.

DISTRIBUTION.[3]—The white-pine shoot borer has been reported from points in Ontario and Quebec, Canada; and in Connecticut, Maine, Massachusetts, New Jersey, New York, Pennsylvania, and Wisconsin in the U.S.A.

Pennsylvania collections have been made in the following counties: Berks, Fulton, and Schuylkill. In addition, symptoms of attack were found in these counties: Clearfield, Crawford, Cumberland, and Perry.

DAMAGE.—The larva hollows the pith of newly developing terminal shoots and cuts most of the new wood around the inner bark at the base of its tunnel (figs. 2 & 3). This damage causes the new shoot to fade, then turn red as summer progresses. Eventually the attacked leaders and some laterals break where the wood had been cut, leaving a short stub of sound material (fig. 4). This injury may be of little concern on laterals, but the high incidence of leader attack on Scotch pine is a serious problem to the Christmas tree growers. The tree may be left with a 2- to 4-inch terminal instead of the 8- to 12-inch size growers prefer. An occasional stout Scotch pine leader may resist breakage, but frequently these become distorted. Attacks on small-diameter shoots may result in starvation of larvae, and these shoots turn red early in the season (mid-June). Such shoots do not show the typical severance, but the wood becomes pitch soaked.

LIFE HISTORY.—Occasional trips to the DeLong plantation at Knauers were made in 1957, 1958, 1959, and

[3] J. F. Gates Clarke, U. S. National Museum, kindly gave these records, except for Ontario and New Jersey.

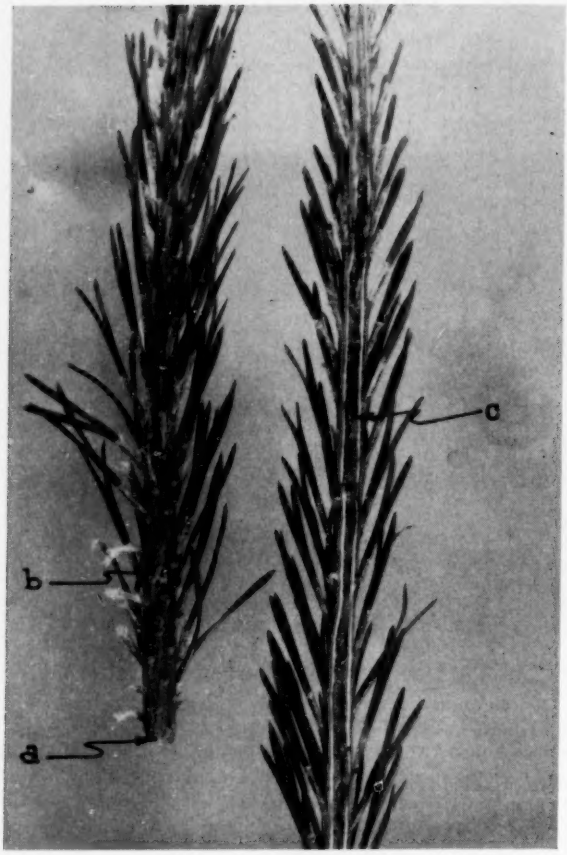

FIG. 2.—Scotch pine leaders showing: (a) cut end, (b) larval exit, and (c) *Eucosma gloriola* larva in frass-filled burrow.

FIG. 3.—Douglas-fir leader cut to show *Eucosma gloriola* larva *in situ*.

observations of the stages present were recorded. Although only a limited amount of information was obtained in this fashion, it has been useful since little could be found in the literature.

Infested shoots were collected in mid-June to rear the moths and any parasites. The insect is univoltine and requires several months at near freezing temperatures to break pupal diapause.

Moths were seen for the first time on May 6, 1959. They were resting on shaded Scotch pine needles at 2:30 P.M., E.D.T., in a normal position with their heads facing the twig. At the same time early-instar larvae were found in the pith of a few shoots. Evidently moths began emerging in late April 1959, soon after the buds burst. At this season they are well camouflaged when they oviposit on the new shoots. The color of the tiny moths blends perfectly with that of the sloughed bud scales.

The larva mines the pith from a few inches above the node of the new shoot to near the tip. The larva feeds up and down in the burrow and eventually cuts the woody portion of the stem. Sometimes several larvae develop within a shoot.

That the older larvae cut the shoots is apparent. Brushing a gloved hand over the upper terminals of Scotch pine in mid-May gave little evidence of *Eucosma* attack. Use of the same technique on June 4, 1959, indicated 26% of the trees were infested, and by June 16, 50% of the trees had shoots that snapped.

In 1957 most larvae were estimated to be in their penultimate or ultimate instar on June 10, and pupation was about complete in the laboratory on June 24. Next season the cold wet weather delayed activity so that the late-instar larvae were still common on June 24, although some larvae pupated by June 22, and a few larvae were present on July 6 in the field. The spring weather of 1959 was about normal, and an examination of shoots indicated that 2% of the insects had cut exit holes and had left their chambers by June 16.

Pupation occurs in a loosely woven light brown cocoon in the upper layer of debris or soil. Soil particles adhere to the cocoons, making them difficult to find. The insect remains a pupa from June or early July until late next April or early May. Prior to emergence of the moth, the pupa forces its way through the cocoon aided by backward-directed spines. The thorax splits dorsally and the moth appears.

CONTROL.—Control with insecticides or other means has not been attempted. Possibly high volume or concentrated DDT sprays would give satisfactory control if applied to the upper branches about May 1 and again on May 10 in southern Pennsylvania. Granulated insecticides, either contact or systemic type, should be effective

when applied to the soil to kill larvae dropping to pupate or to feed. It is doubtful that an attempt to clip and destroy infested shoots would be successful.

An ichneumon, determined by L. M. Walkely of the U. S. Department of Agriculture, Entomology Research Division as *Glypta* n. sp. parasitized 61% of the larvae in 1958 and 56% in 1959. The sex factor for 51 parasites in 1958 was 0.67. This *Glypta* overwinters as a mature larva within its cocoon inside the shoot borer cocoon.

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FIG. 4.—Scotch pine sapling showing leader that had been attacked by *Eucosma gloriola*, and broken by wind at the point where the larva had severed the wood.

Hyperodes humilis, a New Pest of Sweet Corn in the Everglades, and Its Control[1]

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ABSTRACT

Hyperodes humilis (Gyll.) grubs found mining the lower stems of young sweet corn plants in the Everglades during the spring and fall of 1959 seemed to cause the plants to become stunted, break-over, wilt, or die. This insect was not previously known to attack cultivated crops. Best control was obtained with DDT at 2 pounds or Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate) at 1 pound of actual toxicant per 100 gallons, beginning on the day of seedling emergence and continuing at 4-day intervals for approximately 1 month. Special effort should be made to direct the spray to the lower stem and surrounding soil. Adults feed on the stem and foliage of young plants, but this feeding seems to cause little damage.

During February 1959 small weevil grubs were discovered mining stems of sweet corn plants near the soil surface (Harris 1959). Adults of this insect were first identified as *Hyperodes delumbus* (Gyll.). Adult specimens were identified by Dr. E. L. Sleeper, Long Beach State College, Long Beach, California, as *Hyperodes humilis* (Gyll). This identification was confirmed by Miss Rose Ella Warner of the Entomology Research Division, U. S. Department of Agriculture. *H. humilis* grubs were active

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FIG. 1.—Damage to sweet corn plants by grubs of *Hyperodes humilis* (Gyll.).

on young sweet corn plants during the spring growing season and appeared in heavy numbers on sweet corn planted in the fall of 1959.

CROP DAMAGE.—The grub burrows aimlessly within the stem near the soil line, either above or below the soil surface. These mines may be near the surface of the stem or extend to the middle; when near the surface the mine resembles leaf miner feeding on other crops (fig. 1). Infested plants may be stunted, break-over or lodge (especially in winds and rain), or wilt. Death of the young plant may often follow wilting.

Although direct cause and effect has not been shown experimentally, this plant damage seems to be caused by the grubs. Plants about 1 month old seem to be less susceptible to damage and less attractive to ovipositing females than older plants. On the basis of the feeding habits and damage caused by the grubs it seems appropriate to call this insect the corn stem weevil.

LITERATURE.—Dietz (1889) and Blatchley & Leng (1916) described the adult of *H. humilis*. Blatchley and Leng report the range as "from Newfoundland and Quebec to Montana, south to Kansas and Mississippi." The Florida State Plant Board reported in personal correspondence that the insect was not reported in Florida prior to its discovery on sweet corn in the Everglades in February 1959.

H. humilis belongs to the tribe Hyperini of the Curculoinidae, which also contains the alfalfa weevil, *Hypera postica* (Gyll.), and the vegetable weevil, *Listroderes costirostris obliquus* (Klug). Although *H. humilis* has not been reported previously to feed on economic plants, occasional references to plant damage by other species of *Hyperodes* have been made. *Hyperodes* sp., probably *porcellus* Say, attacked lawn grass roots in Connecticut (Britton 1932). In New Zealand, *H. griseus* Hust. was reported to be a pest of wheat, rye grass (*Lolium*), barley (Morrison 1938), and crested dog's-tail, *Cynosurus cristatus* L. (Jacques 1940). The described damage to wheat was similar to that to sweet corn by *H. humilis*. Essig (1926) reported an incidence of *H. hyperodes* (Dietz) boring into the stems of young tomato plants and onions in California in 1923. Jones (1954) reported finding live larvae of *Hyperodes* sp. in carrots from Argentina, while Beauchamp (1954) and Crowell (1954) reported them in carrots from Uruguay. Both finds were in ships' stores at Boston.

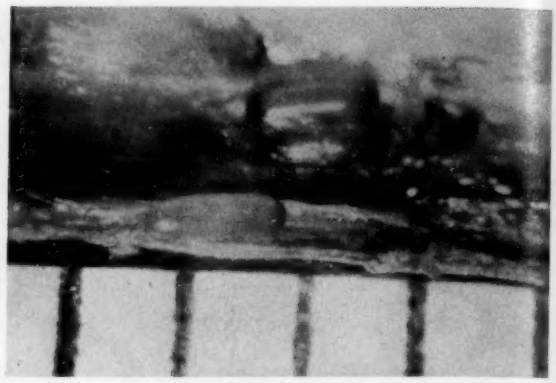

FIG. 2.—Eggs of *Hyperodes humilis*. Scale equals 1/32 inch.

DESCRIPTION AND DURATION OF LIFE STAGES.—There is apparently no literature on the life history or description of immature stages of *H. humilis*. In reference to the genus, Blatchley & Leng (1916) reported "they occur beneath cover on sandy or muddy ground near water, or upon semi-aquatic plants. They probably feed upon such plants, though the life history of no species is recorded. Up to the present they may, therefore, be termed innocuous rather than injurious to agriculture." Life-history studies were made from April through July.

Eggs are imbedded in the coleoptile or the sheaths of the lower leaves of the young corn plant and are more easily detected on the under or inner surface of these plant parts. They are creamy white when first laid but in a day or two change to dark brown. They are in the shape of a cylinder with hemispherical ends and are about 1/32 inch long and 1/96 inch in diameter (fig. 2). Twenty-six eggs placed on moist filter paper in Syracuse watch glasses hatched 3 to 6 days after oviposition (table 1).

Grubs are 1/32 inch long when first hatched and about ¼ inch long when mature. The head is dark brown and the legless body is translucent white. White hairs extend laterally and caudally from the body. Grubs are shown

Table 1.—Length of life stages of *Hyperodes humilis*.

EGG STAGE		LARVAL STAGE		PUPAL STAGE	
No. of Individuals	Days	No. of Individuals	Days	No. of Individuals	Days
1	3	3	20	2	4
6	4	1	21	6	5
12	5	1	25	2	6
7	6			1	9
Average	4.9		21.2		5.4

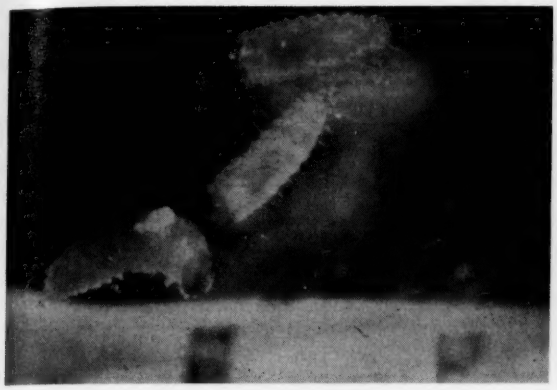

FIG. 3.—Young grubs of *Hyperodes humilis*. Scale equals 1/16 inch.

in figure 3. Among five individuals reared in corn stalk sections, the length of the larval stage was 20 to 25 days (table 1).

Pupae are white, about 5/32 inch long, and typically curculionid in appearance (figure 4). The duration of this stage ranged from 4 to 9 days for 11 individuals (table 1). No pupae have been found in the field, but in the laboratory pupation occurred in sections of corn stalk.

Adult weevils are about 5/32 inch long, and dark brown with white scales (figure 5). They are more completely described by Dietz (1889) and by Blatchley & Leng (1916). No information has been reported concerning the time elapsing between emergence and mating or between mating and oviposition.

Although adults have occasionaly been found above the soil surface during the day they seem to be more active at night. Adults have been collected from stem and foliage of sweet corn plants and at house lights at night. Berlesse funnel collections indicated that adults remain in the soil near young corn plants during daylight hours. From 99 soil cores (2.75 inches in diameter and 8 inches deep) surrounding individual young corn plants, 26 adult weevils were collected in Berlesse funnels. The length of the life cycle of the few specimens of *H. humilis* under study was approximately 1 month.

HOST PLANTS.—As the species has been known to occur in this country for many years with no record of being a pest of economic crops and is present in corn fields in large numbers, it is probable that at least one undetected natural host plant is present in the Everglades. The species probably existed on this host plant for several years before individuals began to oviposit on sweet corn (at least in sufficiently large numbers to be noticed). In addition to sweet corn, grubs have been found in sorghum, *Sorghum vulgare* Pers., and field corn. Eggs have been found on nutgrass, *Cyperus rotundus* L., and goosegrass, *Eleusine indica* (L.) Gaertn., in corn fields.

CHEMICAL CONTROL.—Observations in commercial sweet-corn fields indicated that budworm control programs using toxaphene, DDT, heptachlor, or mixtures of these chemicals with parathion seemed to be ineffective in controlling *H. humilis*. In a budworm control experiment in which weekly applications were begun about 1 week after seedling emergence, toxaphene and DDT at dosages as high as 2 pounds of actual toxicant per 100 gallons did not cause noticeable reduction in the amount of damage caused by *H. humilis*.

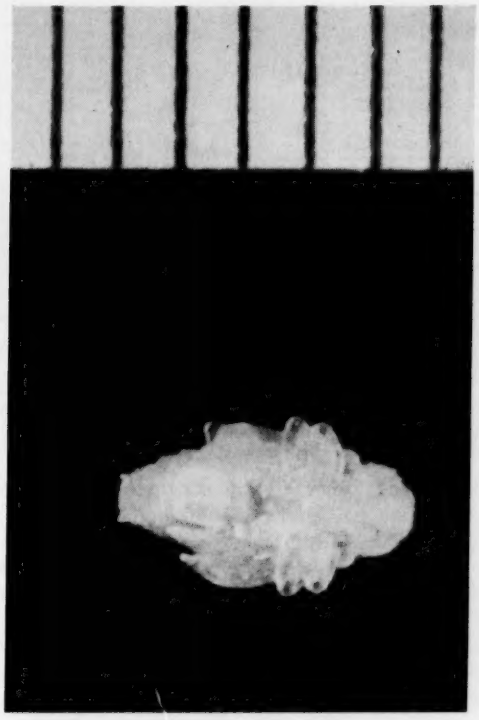

Fig. 4.—Pupa of *Hyperodes humilis*. Scale equals 1/32 inch.

In each experiment sweet corn was planted in rows 36 inches apart. Each plot was four rows wide and separated from adjacent plots on each side by a single unsprayed buffer row. Sprays were applied with a custom-built, self-propelled, small-plot sprayer (Harrison *et al.* 1958) equipped with a two-row outrigger boom and driven over the unsprayed buffer rows. All insecticides were applied in emulsions.

Treatment means were compared by analysis of variance and the Student-Newman-Keuls test (Federer 1955). Where vertical lines appear in the figures and tables, they indicate significant differences. Treatments that are joined by a common line are not significantly dif-

FIG. 5.—Adult of *Hyperodes humilis*.

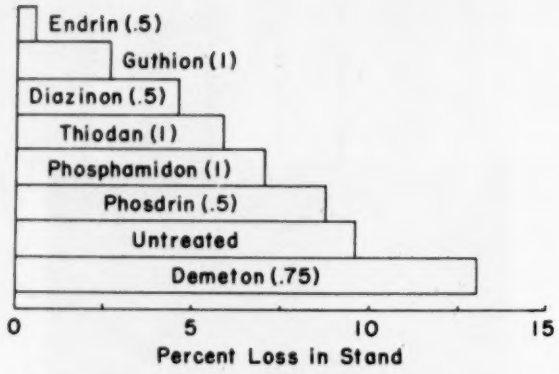

FIG. 6.—Multiple postemergence insecticide applications and per cent loss in stand in Experiment I.

ferent from each other; treatments that are not joined by a common line are significantly different.

Experiment I.—The purpose of this experiment was to determine the effectiveness of preplanting broadcast soil treatments and to screen several insecticides in multiple postemergence sprays.

Three preplanting broadcast soil treatments and an untreated check were replicated twice in each of four tiers of plots that were 15 feet wide and 50 feet long. Each of the seven multiple postemergence treatments and the corresponding untreated check were applied to one plot in each tier. A semi-latin-square design was used so that each postemergence treatment followed each preplanting broadcast treatment once and only once.

Heptachlor, parathion, and a heptachlor (50%)-parathion (50%) mixture were applied at 5 pounds of actual toxicant per acre in emulsions and immediately disked under to a depth of 6 to 8 inches.

Sixty-Pak sweet corn was planted March 5, 1959. After seedling emergence, 20-foot-wide alleys were disked across the planting to form four tiers with 30-foot rows.

Postemergence sprays were directed to the soil and the lower part of the corn stem. The insecticides used and the amount of actual toxicant applied per 100 gallons are shown in figures 6 to 8. All sprays were applied through Spraying Systems D2-25 Teejet nozzles at 250 p.s.i. with the sprayer traveling approximately 2.4 m.p.h. The first

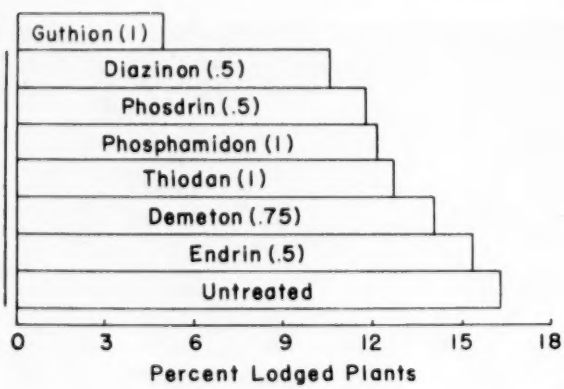

FIG. 7.—Multiple postemergence insecticide applications and per cent lodged plants in Experiment I.

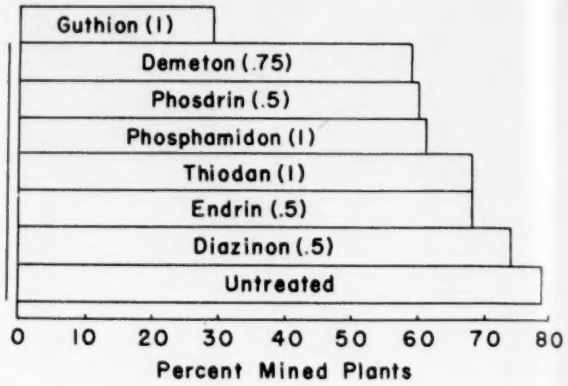

FIG. 8.—Multiple postemergence insecticide applications and per cent grub-mined plants in Experiment I.

spray was applied March 11 when the first seedlings emerged. Additional sprays were applied March 23, March 31, and April 7. Two overhead nozzles per row were used in the first two applications to apply approximately 50 gallons per acre. Because the corn foliage seemed dense enough to prevent spray from hitting the stem and adjoining soil, a nozzle was added on each side of the row for the two final sprays. The rate of application was approximately 100 gallons per acre.

Plants in the two middle rows of each plot were counted March 23 and April 15. On April 2 heavy winds and rains caused some of the mined plants to lodge or break-over. On each plant that was examined the break was at the site of a mine. The total number of plants and the numbers of lodged plants in the two middle rows of each plot were recorded on April 3. On the same day, 10 plants were taken from each of the outside rows of each plot and examined for mines.

Preplanting broadcast applications did not have a significant effect on per cent reduction in stand or upon per cent lodged plants (table 2). The Student-Newman-Keuls test indicated that the percentage of mined plants was significantly higher in the plots that were treated before planting with parathion or the parathion-heptachlor mixture than in untreated check plots.

Multiple postemergence applications did not have a significant effect on per cent reduction in stand from March 23 to April 15 (figure 6). Neither was there a significant effect on actual stand on either date. Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate) at 1 pound of actual toxicant per 100 gallons resulted in a significantly lower percentage of

Table 2.—Control of *Hyperodes humilis* with preplanting broadcast soil applications in Experiment I.[a]

Insecticide	Per Cent		
	Reduction in Stand	Lodged Plants	Mined Plants
Untreated	3	12	53
Heptachlor	10	11	59
Heptachlor+parathion	6	11	67
Parathion	7	14	69

[a] Means joined by the same line are not significantly different; others are.

lodged plants (figure 7) and mined plants (figure 8) than the other insecticides. Although they were used at dosages much higher than commonly used in postemergence or foliage applications, the other insecticides failed to have any significant effect on reducing the incidence of attack or damage caused by *H. humilis* grubs.

Failure to control *H. humilis* with preplanting broadcast soil applications could have been caused by inadequacy of the method or the ineffectiveness of the particular insecticides used. The higher incidence of infestation following preplanting treatments of parathion and the heptachlor-parathion mixture could have been chance variation or caused by some other factor, such as the destruction of natural enemies. Although the treatments did not differ significantly from each other or from the untreated check in their effect on stand reduction, Guthion (the only effective insecticide by other criteria) resulted in a percentage of stand reduction that was much less than that in the untreated check or in some of the other treatments. Endrin resulted in a lower stand reduction than Guthion, but gave little reduction in mined or lodged plants.

Experiment II.—The purpose of this experiment was to reevaluate Guthion at two dosages and to evaluate several insecticides not included in the first trial. The insecticides used and the amount of actual toxicant applied per 100 gallons are shown in figures 9 to 11.

Golden Security sweet corn was planted April 6, 1959.

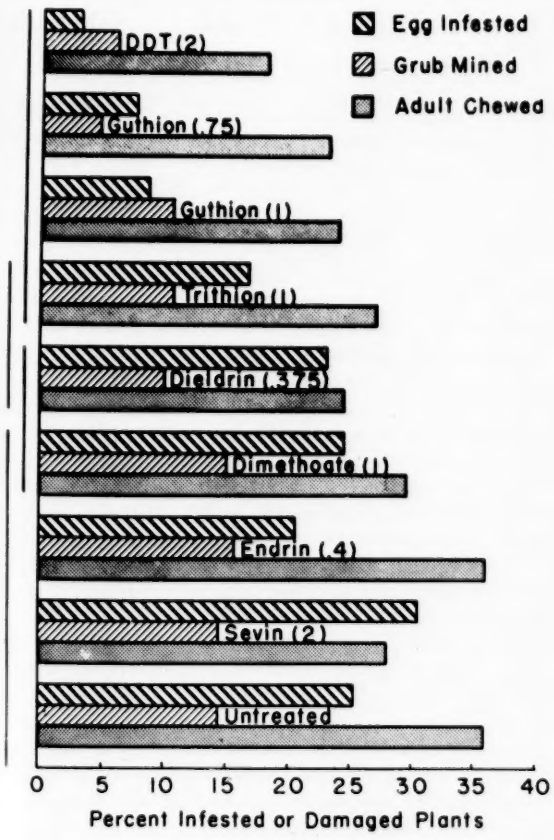

FIG. 9.—Insecticides and per cent infested or damaged plants following the third spray in Experiment II.

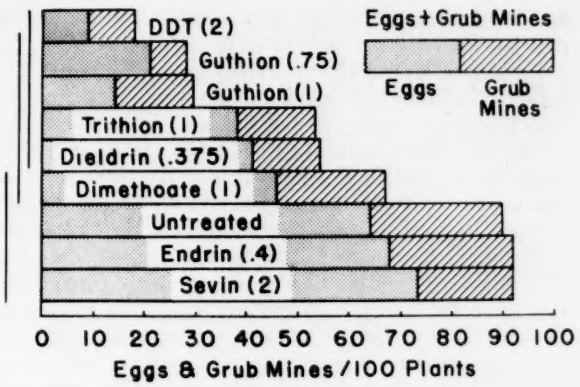

FIG. 10.—Insecticides and numbers of eggs and grub mines per 100 plants following the third spray in Experiment II.

Each treatment was replicated seven times in a randomized complete block design. Each plot was 25 feet long; blocks were separated by 20-foot alleyways.

Seven applications were made at 4-day intervals beginning April 11, when the first seedling appeared. Spray applications were made with Spraying Systems D2-25 Teejet nozzles and at a pressure of 250 p.s.i. with the sprayer traveling at approximately 2.4 m.p.h. Approximately 50 gallons per acre were applied through two overhead nozzles in the first three applications. For the remaining applications a nozzle was added on each side of the rows to direct the spray under the foliage to the soil and lower stem. This addition increased the rate of application to approximately 100 gallons per acre.

Plants in each plot were counted April 21 and May 5. These counts did not show any significant differences among the treatments in their effect on stand or the per cent reduction in stand between the two dates. The per cent reduction in stand was only 4.7 in the untreated check.

On April 20, one day after the third spray, five plants were taken at random from each plot row and examined to determine the per cent infested with eggs, mined by grubs, and chewed upon by adults. Also, a count was made of the total numbers of eggs and mines in each plot sample. The sheaths of the two lower leaves and the coleoptile were stripped from the plant and the inner sur-

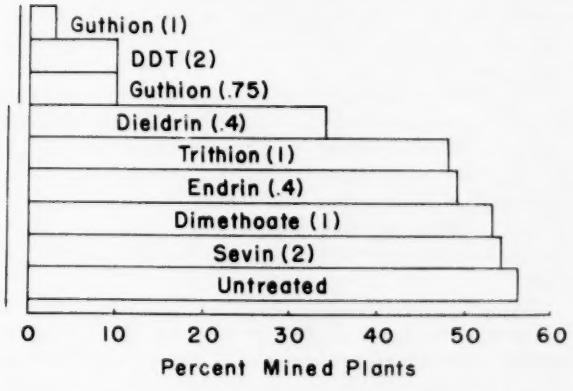

FIG. 11.—Insecticides and per cent grub-mined plants following the sixth spray in Experiment II.

face of these parts and the stem were examined under a dissecting microscope (9×) to make the counts.

On May 4, 3 days after the sixth spray, 25 plants were taken at random from the 2 middle rows and examined with the naked eye to determine the per cent mined.

One day after the third spray, DDT and each rate of Guthion resulted in a significantly lower percentage of plants affected by *H. humilis* than resulted from application of all the insecticides other than Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) (fig. 9). The other chemicals were little better than the untreated check. The effective chemicals seemed to be much more effective in reducing egg infestation and grub damage than in reducing the amount of adult chewing. DDT and Guthion, at both rates applied, were the most effective chemicals on the criterion of numbers of eggs and mines per 100 plants (fig. 10).

On the third day after the sixth application, DDT and Guthion treatments had a significantly lower percentage of mined plants than the untreated check or the other treatments. The other insecticides were not significantly better than the untreated check.

Experiment III.—The purpose of this experiment was to evaluate Guthion at a rate that is more common for use in the control of agricultural pests and to determine the effect of gallonage and spray adjuvant on the efficacy of this insecticide. It was planned to apply the same amount of actual Guthion per acre in 50 and 200 gallons per acre. At each gallonage there were three treatments: Guthion alone, Guthion plus Triton X-100[2], and Guthion plus Plyac[3] spreader-sticker. Triton X-100 and Plyac were used at one-half pint per 100 gallons. Guthion was used at dosages of 6 and 1.5 ounces per 100 gallons in the low and high gallonages, respectively.

Golden Security sweet corn was planted April 6, 1959. Each treatment and the untreated check were replicated seven times in a randomized complete block design. Plots were four rows wide and 25 feet long. Spray applications were begun April 11 when the first plants emerged and were continued for seven applications at 4-day intervals.

Sprays were applied at 50 p.s.i. with the sprayer traveling approximately 1.8 m.p.h. Spraying Systems Teejet nozzles were used, No. 6502 nozzles for the low gallonages and No. 6508 for the high gallonages. Two nozzles per row were used throughout the experiment. For the first three sprays they were placed over the rows; for the next four sprays they were placed on drops at the sides of the rows to direct the spray to the lower stem and soil surface below the foliage canopy.

Theoretically the 6508 nozzles should have put out four times as much spray as did the 6502 nozzles, but the output of the former was much less than expected. The average rate of application for low gallonage sprays was approximately 45 gallons of spray, or 0.17 pounds of actual Guthion per acre; for the high gallonages, about 127 gallons of spray or 0.12 pounds of actual toxicant per acre.

On April 29, 2 days after the fifth spray, 25 plants were taken from each row of the four row plots and examined under a two-power reading glass between April 29 and May 4 to determine the per cent infested with eggs, mined by grubs, and chewed upon by adults. The sheaths of the two lower leaves were removed and examined from the inner surface. Plants were held at 40° F. until they could be examined.

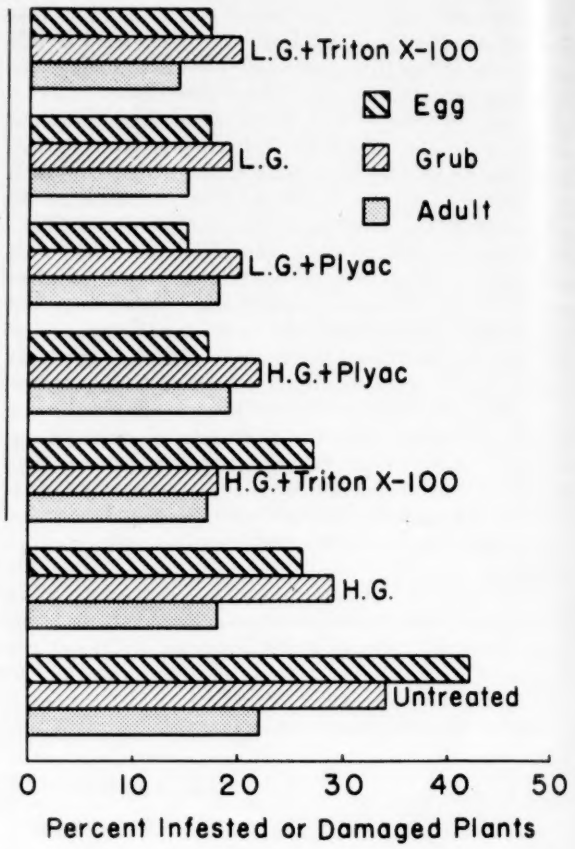

FIG. 12.—Effect of low gallonage (L.G.), high gallonage (H.G.) and spray adjuvants on the control of *Hyperodes humilis* with Guthion.

Counts of the stand were made April 21 and May 5. No significant differences were evident among the treatments or between treatments and the untreated check on the criteria of reduction of stand. The reduction of stand in the untreated check was only 2.4%.

The two gallonages were not significantly different from each other in reducing the incidence of *H. humilis* infestation. All the Guthion treatments significantly reduced the incidence of infestation (fig. 12). The other treatments were significantly better than the high-gallonage treatment in which no spray additive was used. Treatments had a greater effect on the per cent of egg-infested plants and of mined plants than they did on the per cent of plants chewed upon by adults.

Conclusions.—Preplanting soil treatments of heptachlor, parathion, or a heptachlor-parathion mixture failed to reduce the incidence of *H. humilis* attack on sweet corn.

Of 12 insecticides that were tested at abnormally high dosages, only DDT and Guthion gave any substantial reduction of *H. humilis* attack. Comparison of criteria on eggs, mines, and adults indicated that the chemicals

[2] Trademark of Rohm & Haas Company.
[3] Trademark of Allied Chemical & Dye Corporation.

probably killed the adult insect before oviposition, although adult feeding was not greatly reduced. DDT at 2 pounds or Guthion at 1 pound per 100 gallons beginning at emergence and continued at 4-day intervals for approximately 1 month should give a high degree of control of this insect, if special care is taken to direct the spray at the lower part of the stem and the soil. Guthion is not available for commercial use on sweet corn at present.

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Biology of the Convergent Lady Beetle When Fed a Spotted Alfalfa Aphid Diet[1]

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ABSTRACT

Laboratory studies on the biology of the convergent lady beetle (*Hippodamia convergens* G.-M.), when fed spotted alfalfa aphids (*Therioaphis maculata* (Buckton)), showed that the incubation period of the egg averaged 2.0 days, the larval stage 7.6 days, the pupal stage 4.1 days and the period from egg to adult 13.8 days. There were four larval instars. Daily consumption of aphids per instar was in direct arithmetical proportion and total consumption in direct geometrical proportion to the larval instar. When reared in the laboratory adult males lived longest on a diet of 60 and females on 90 aphids per day, whereas field-collected adults of both sexes lived longest on 30 aphids per day. Most laboratory-reared adults lived longer than field-collected adults. Females from both sources generally lived longer than males. The length of the immature stages was shorter and daily aphid consumption higher than when other aphids were used as the diet.

Since the spotted alfalfa aphid (*Therioaphis maculata* (Buckton)) has been found in Arizona, the convergent lady beetle (*Hippodamia convergens* G.–M.) has increased in its importance as a predator of this pest. The beetle is most abundant in the spring and more effective during that period than any other predator studied in Arizona.

Several workers in other States (Bieberdorf & Bryan 1956, Davis *et al.* 1957, Dickson *et al.* 1955, Smith & Hagen 1956, and Tuttle 1956) have indicated the convergent lady beetle's abundance and feeding efficacy over other aphid predators. Recently it has been found to consume twice as many spotted alfalfa aphids as does, its nearest competitor, *Collops vittatus* (Say), (Nielson & Henderson 1959).

In the summer of 1958 a laboratory study was made of the biology of this lady beetle when fed a diet of spotted alfalfa aphids. This study was started in Tempe, Arizona, and completed in Mesa. Special emphasis was placed on aphid consumption by the larvae and on the longevity of adults. Comparative data for other aphid species reported in the literature are also included.

LENGTH OF IMMATURE STAGES.—Beetles were collected in the field, and pairs of adults were confined in plastic containers for mating. After the eggs hatched the larvae were placed individually in 1-×5-inch glass tubes stoppered with cork disks, and were fed spotted alfalfa aphids daily until the end of the last instar. The lengths of the egg, larval, and pupal stages are given in table 1. About 14 days were required for the insect to complete its development.

APHID CONSUMPTION.—Records were kept on the number of spotted alfalfa aphids consumed by the larvae each day. The results are summarized in table 2. The data indicate that the daily consumption per instar was in direct arithmetical proportion and the total consumption in direct geometrical proportion to the instar. By analogy, these data may lend support to hypothetical growth curves for other sap-sucking insects. Such data are difficult to obtain, owing to lack of techniques for accurate

[1] Accepted for publication October 12, 1959.

[2] In cooperation with the Arizona Agricultural Experiment Station.

Table 1.—Length, in days, of immature stages of the convergent lady beetle in the laboratory, June 13–27, 1958, Tempe, Arizona.[a]

Stage	Range	Average
Egg	2–2	2.0
First instar	1–2	1.3
Second instar	1–2	1.3
Third instar	1–3	1.8
Fourth instar	3–4	3.3
Pupa	3–4	4.1
Total	11–18	13.8

[a] 45 records for all stages except egg, 50.

Table 2.—Consumption of spotted alfalfa aphids by larval instars of the convergent lady beetle, June 16–27, 1958, Tempe, Arizona.

Instar	No. of Aphids per Beetle	
	Daily	Total
First	16.9	22.9
Second	23.6	29.9
Third	44.3	78.7
Fourth	63.1	206.2

measurement of amounts of sap consumed by these insects.

Adult Longevity.—Information on adult longevity of the convergent lady beetle was obtained from laboratory-reared and field-collected adults. Twenty-five adult beetles of each sex from each source were placed individually in glass tubes similar to those used for the larvae, and arranged in groups of five. The first group received no aphids and each of the other groups received a different number of aphids per day. The longevity of each group is shown in table 3. Males reared in the laboratory lived longest on 60 aphids and females on 90 aphids per day. The loss of one female after the 22nd day shortened the overall longevity of the 60-aphid-per-day group. Both sexes from the field lived longest on a diet of 30 aphids per day. In general laboratory-reared aphids lived longer than those from the field. Females from both sources generally lived longer than males. The adults that were starved lived only a few days.

Table 3.—Longevity, in days, of adult laboratory-reared and field-collected convergent lady beetles after feeding on a constant diet of given numbers of spotted alfalfa aphids, June 26 to August 18, 1958, Mesa, Arizona.

No. of Aphids Fed Daily	Males		Females	
	Laboratory	Field	Laboratory	Field
120	23.0	14.0	26.3	22.8
90	31.2	23.8	32.0	6.8
60	38.8	20.6	27.2[a]	27.8
30	17.6	29.2	27.8	33.6
0	2.0	1.0	2.2	3.2

[a] One lost on 22nd day.

The greater longevity of laboratory-reared individuals indicated that the adults from the field were at least 7 days old at the time they were collected. Moreover, the greater food requirements for the laboratory-reared adults indicate prior feeding by the field-collected group.

Comparative Data on Other Aphid Species.—The value of the convergent lady beetle as a predator of other aphid species has been recognized by other workers. Table 4 shows the length of the immature stages and consump-

Table 4.—Length of immature stages and consumption of various aphid species by the convergent lady beetle in different States, as reported by several workers.

State and Species	Literature Source	Length of Immature Stages (Days)			Number of Aphids Consumed	
					Average per	
		Egg	Larva	Pupa	Larval Stage	Adult per Day
Arizona						
Therioaphis maculata	Nielson & Henderson (1959)	—	—	—	—	97
	Present paper	2	8	4	—	—
California						
Macrosiphum pisi	Campbell (1926)	—	—	—	200	20
Phorodon humuli (Schr.)	Clausen (1915)	5	16	7	349	56
Colorado						
Chaitophorus negundinis (Thomas) *Macrosiphum gaurae* Williams *Macrosiphum rosae* (L.) *Prociphilus fraxinifolii* (Riley)	Palmer (1914)	3	14	5	433	67
Florida						
Aphis spiraecola Patch	Miller & Thompson (1927)	4	11	5	—	—
	Thompson (1928)	—	—	—	616	87
Minnesota						
Toxoptera graminum (Rond.)	Stehr (1930)	—	—	—	124	46
Oregon						
Macrosiphum pisi	Rockwood (1952)	—	—	—	207	10

tion of various aphid species. All the studies were conducted while the beetles were in captivity. The shortest length of life was in Arizona, and the longest in California. The differences appear to be due to environment and food. Daily aphid consumption also varied considerably. It was lowest in Oregon and California with *Macrosiphum pisi* (Harris), and highest in Arizona with *Therioaphis maculata*.

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Distribution and Sorption of Liquid Fumigants Applied to Wheat by Recirculation[1]

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ABSTRACT

Laboratory fumigation experiments with wheat at 12.2% moisture and 77° F. were conducted in recirculators using a liquid formulation of 76.5% carbon tetrachloride (CCl_4), 3.5% ethylene dibromide (EDB), 10% carbon disulfide (CS_2), and 10% ethylene dichloride (EDC), by weight, to study the sorption and vertical distribution of each component. Gas samples were taken at five levels and five times during fumigation and were analyzed by mass spectrometry and by thermal conductivity. A small amount of EDB reached the bottom, but nearly all the EDB apparently was sorbed in the upper levels during the 30 minutes of recirculation. The other components were fairly evenly distributed but showed a slight tendency to settle downward during the longer exposures. Selective sorption occurred and the decreasing order of sorption was EDB, EDC, CS_2, CCl_4. Based on pounds of fumigant per 1000 cubic feet, the average composition in gas samples taken from interstitial space after 24 hours of exposure was 86.56% CCl_4, 0.00% EDB, 7.15% CS_2, 1.26% EDC, and 5.24% CO_2. Of the total formulation about 65% and 85% were sorbed during ½ and 24 hours, respectively.

The recirculation method of fumigating bulk-stored grain offers many advantages over the conventional methods, which utilize the forces of nature to distribute the fumigant vapors in a grain mass. Phillips (1957) was among the first in this country to report the practicality of applying liquid fumigants by recirculation. Considerable information is available concerning the insecticidal performance of liquid fumigants, but relatively little is known about the distribution and sorption of each chemical component. Kenaga (1956) reported on the gravity distribution of the components of liquid fumigants in a column of grain. The present paper presents the results of experiments conducted to determine the distribution and sorption of four chemical compounds in a liquid formulation when applied by the recirculation method.

METHODS AND MATERIALS.—Serafume®[4], a fumigant formulation containing 76.5% carbon tetrachloride (CCl_4), 3.5% ethylene dibromide (EDB), 10% carbon disulfide (CS_2), and 10% ethylene dichloride (EDC), by weight, was selected because it provided an opportunity to observe four of the commonly used components of commerical mixtures in one experiment. Duplicate fumigations were conducted on two different dates, making a total of four replicates. The average fumigation temperature was 77° F. (74° to 80°).

Each recirculator (Fig. 1) was 6 feet tall and 8 inches in diameter and was filled with 100 pounds of Hard Red Winter Wheat having a moisture content of 12.2%. The total volume of each recirculator, including the blower and duct, was 2.47 cubic feet. Copper gas sampling tubes were located at the 3-, 18-, 36-, 54-, and 72-inch levels, from the top downward. After filling the recirculators with wheat and sealing them with plastic tape, a neoprene stopper was removed from the top of each and the fumi-

[1] Accepted for publication October 14, 1959.

[2] Resigned October 1, 1958; now instructor and assistant entomologist, Kansas State University, Manhattan.

[3] One of the field stations of the Stored-Product Insects Section, Market Quality Research Division, U. S. Department of Agriculture.

[4] Trademark of the Dow Chemical Company. The use of trade names in this paper is for identification purposes only and does not constitute an endorsement of the products by the United States Department of Agriculture.

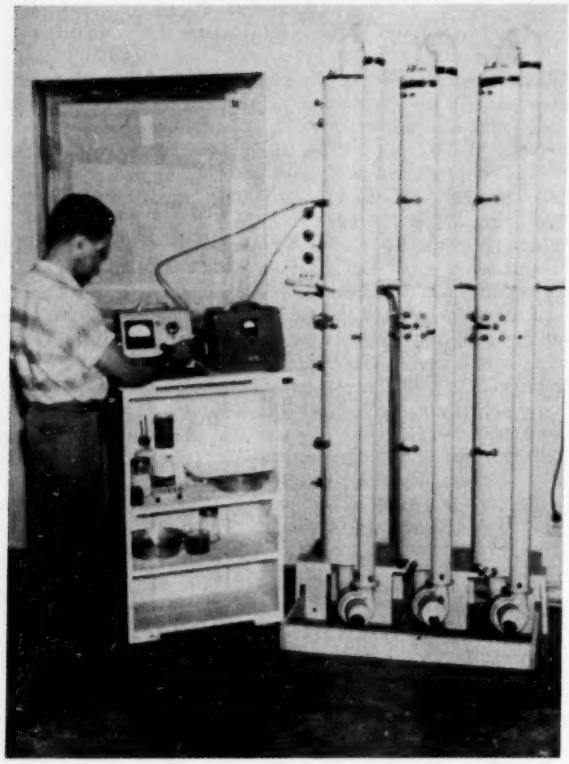

FIG. 1.—Recirculation tests were conducted in these three cylinders which are equipped with blowers to move air downward through the larger column and return it upward through the smaller pipe.

gant was applied to the wheat surface by means of a hypodermic syringe. The dosage rate was 2 gallons per 1000 bushels. When calculated on the basis of the pounds of each component per 1000 cubic feet of free space the dosages were: CCl_4, 22.74 lbs.; EDB, 1.04 lbs.; EDC, 2.97 lbs.; and CS_2, 2.97 lbs. Immediately after application of the fumigant, the stopper was replaced and the recirculation started. Each fan moved air at the rate of 0.82 cubic feet per minute per bushel and was operated for 30 minutes. This movement made a total of about 30 air changes in the wheat.

Gas samples were drawn and analyzed by a Gow-Mac, 4-filament, air reference, thermal conductivity (T/C) gas analyzer and returned to the same level from which they were drawn. Samples were taken 30 minutes, 1 hour, 4 hours, 8 hours, and 24 hours after the beginning of the tests. In addition to the Gow-Mac analyses, an additional 125-ml. gas sample was taken from each level at each of the time intervals for mass spectrometric analyses by the Dow Chemical Company. Special care was taken so that the samples were not diluted by air in the sample lines. The sensitivities, or error limits, for measurement of air-fumigant mixtures analyzed by mass spectrometry were as follows:

Compound	*Molar per cent*	*Pounds per 1000 cu. ft.*
Ethylene dichloride	0.005	0.014
Ethylene dibromide	0.005	0.026
Carbon disulfide	0.002	0.004
Carbon tetrachloride	0.005	0.021

With ethylene dibromide, the lower sensitivity limit is within the range of toxicity to insects. The sensitivity of the Gow-Mac T/C unit was estimated to be 0.05 pounds per 1000 cubic feet for CCl_4, EDC, and CS_2. The T/C unit is very slow to respond to EDB and has very wide limits of error, which were estimated to be in the range of 0.1 to 0.2 pounds/1000 cubic feet.

RESULTS AND DISCUSSION.—Table 1 lists the average concentrations for each combination of time and location. Mean concentrations were also calculated and recorded for all locations combined and each time interval. The sorption and vertical distribution of the components are shown in this table. A small amount of EDB reached the bottom, as indicated in the 30-minute samples, but apparently the bulk of it was sorbed in the upper levels. The amounts of EDB not held by sorption after 1 hour were too small to be measured by this method. The analyses indicated that the other components were fairly evenly distributed by the 30 minutes' recirculation. A slight degree of settling was observed during 24 hours' exposure, probably because the vapors were heavier than air.

It is obvious from this table that the T/C readings gave a more consistent measurement of the total gas concentration than the mass spectrometer totals. However, the T/C readings did not indicate how much of each compound was present. On the average, the T/C readings were 1.4 times the total mass spectrometer readings.

Table 1.—Gas concentrations in wheat-filled recirculators after application of 22.74 lbs. CCl_4, 1.04 lbs. EDB, 2.97 lbs. CS_2, and 2.97 lbs. EDC per 1000 cubic feet free space.

TIME INTERVAL AND DEPTH OF SAMPLE IN INCHES	POUNDS PER 1000 CUBIC FEET[a]						
	Mass Spectrometer						T/C
	CCl_4	EDB[b]	CS_2	EDC[c]	CO_2	Total	Total
30 Minutes							
3	8.98	0.02	0.80	0.28	0.15	10.23	13.29
18	8.52	.01	.72	.26	.15	9.65	12.93
36	9.13	.01	.78	.26	.14	10.32	12.91
54	8.81	.01	.73	.38	.20	10.13	12.84
71	7.24	< .01	.68	.18	.20	8.30	12.81
Mean[d]	8.53	.01	.74	.27	.17	9.72	12.96
1 Hour							
3	6.38	0.00	0.53	0.14	0.18	7.23	11.56
18	5.98	< .01	.56	.14	.18	6.86	11.38
36	6.21	< .01	.49	.12	.18	7.00	11.06
54	6.32	.00	.52	.12	.17	7.13	11.00
71	7.04	.00	.63	.15	.18	8.00	11.87
Mean[d]	6.59	< .01	.55	.13	.18	7.45	11.38
4 Hours							
3	5.50	0.00	0.45	0.11	0.19	6.25	8.66
18	5.70	.00	.44	.09	.17	6.40	8.50
36	4.48	.00	.37	.08	.19	5.12	8.41
54	5.55	.00	.45	.08	.18	6.26	8.78
71	5.08	.00	.47	.10	.18	5.83	9.91
Mean[d]	5.26	.00	.43	.09	.18	5.96	8.85
8 Hours							
3	4.44	0.00	0.33	0.07	0.19	5.03	7.39
18	4.76	.00	.36	.07	.20	5.39	7.13
36	3.32	.00	.30	.04	.20	3.86	7.22
54	5.43	.00	.45	.09	.18	6.15	7.80
71	4.98	.00	.40	.09	.17	5.64	8.63
Mean[d]	4.58	.00	.37	.07	.19	5.21	7.63
24 Hours							
3	3.25	0.00	0.26	0.05	0.20	3.76	5.45
18	3.45	.00	.29	.05	.22	4.01	5.45
36	3.25	.00	.29	.05	.22	3.81	5.59
54	4.00	.00	.32	.05	.22	4.59	5.74
71	4.00	.00	.32	.06	.22	4.60	5.90
Mean[d]	3.59	.00	.29	.05	.21	4.14	5.71

[a] Average of 4 replicates.
[b] Ethylene dibromide.
[c] Ethylene dichloride.
[d] Average of individual samples from all locations at each time interval.

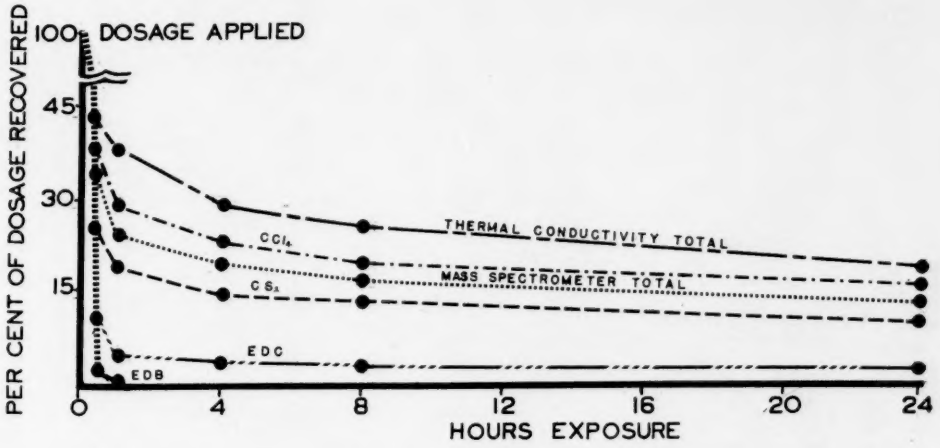

FIG. 2.—Fumigant sorption curves based on amounts recovered from interstitial space in wheat.

Table 2.—Composition of gas samples taken from wheat during fumigation with Serafume.

TIME INTERVAL AND DEPTH OF SAMPLE IN INCHES	PER CENT BY WEIGHT[a]				
	CCl_4	EDB[b]	CS_2	EDC[c]	CO_2
30 Minutes					
3	88.13	0.15	7.85	2.75	1.47
18	88.11	.13	7.45	2.69	1.55
36	89.07	.12	7.61	2.54	1.37
54	88.45	.08	7.33	3.82	2.01
71	87.12	.06	8.18	2.17	2.41
Mean	88.17	.11	7.68	2.79	1.76
1 Hour					
3	88.73	0.00	7.37	1.95	2.50
18	88.86	.06	8.32	2.08	2.67
36	88.71	.04	7.00	1.71	2.57
54	88.64	.00	7.29	1.68	2.38
71	88.11	.00	7.88	1.88	2.25
Mean	88.61	.02	7.57	1.86	2.47
4 Hours					
3	88.85	0.00	7.27	1.78	3.07
18	88.92	.00	6.86	1.40	2.65
36	87.67	.00	7.24	1.57	3.72
54	88.80	.00	7.20	1.28	2.88
71	87.29	.00	8.08	1.72	3.09
Mean	88.31	.00	7.33	1.55	3.08
8 Hours					
3	88.27	0.00	6.56	1.39	3.78
18	88.15	.00	6.67	1.30	3.70
36	86.01	.00	7.77	1.04	5.18
54	88.29	.00	7.32	1.46	2.93
71	88.45	.00	7.10	1.60	3.02
Mean	87.83	.00	7.08	1.36	3.72
24 Hours					
3	86.21	0.00	6.90	1.33	5.30
18	86.03	.00	7.23	1.25	5.49
36	85.30	.00	7.61	1.31	5.77
54	87.72	.00	7.02	1.10	4.82
71	87.53	.00	7.00	1.31	4.81
Mean	87.56	.00	7.15	1.26	5.24

[a] Mass spectrometer.
[b] Ethylene dibromide.
[c] Ethylene dichloride.

This relationship ranged from 0.8 to 2.8. The variations did not indicate any kind of trend which could be correlated with the small degree of selective sorption which occurred after the first 30 minutes and changed the ratios of the components in the interstitial gas.

Table 2 shows the fumigant composition of gas samples taken in each of the different time and location intervals. These data present further evidence of selective sorption in that EDB was sorbed most rapidly and to the greatest extent, followed in order by EDC, CS_2, and CCl_4. The percentage of carbon dioxide was recorded because it produces a T/C signal approximately equal to that of CCl_4. The amount of CO_2 actually remained fairly constant, but as shown in table 2, its relative concentration increased because of the sorption of the fumigant compounds. The selective sorption was very rapid and much of it occurred during the first 30 minutes.

Figure 2 illustrates the relative soption rates of the four components and of the mixture as a whole. Each of the points on the curves was calculated by dividing the average mass spectrometer or T/C reading by the theoretical dosage applied. Again, it is apparent that much of the fumigant (60% to 70%) was sorbed during the first 30 minutes. About 85% of the total applied dosage was sorbed during 24 hours' exposure.

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Laboratory Evaluation of Several Insecticides against *Chrysops* Larvae[1]

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ABSTRACT

Larvae of *Chrysops flavida* var. *reicherti* Fairchild were introduced into solutions or emulsions of several insecticides and held at 23.5° C. for mortality observations. Parathion was the most effective material, causing 100% mortality at 5 p.p.m. Lindane was the outstanding chlorinated hydrocarbon, providing 95% kill at 10 p.p.m.

A program to evaluate insecticides for the control of Tabanidae in the mid-South is being conducted at Stoneville, Mississippi. Most previous investigations have been directed toward control of adults with residual applications to foliage or directly to animals (Howell *et al.* 1949, Brown & Morrison 1955). In general, ineffective results have been reported, although under certain conditions frequent applications of pyrethrum formulated with piperonyl butoxide or butoxypolypropylene glycol have proved of value (Garnett *et al.* 1949, 1955, Goodwin *et al.* 1952, 1953, Bruce & Decker 1955).

Recently Hansens (1956) and Jamnback & Wall (1957) demonstrated good control of larvae of certain salt-marsh *Tabanus* species with dieldrin granules. Lindane was initially effective but short-lived. These reports indicate larval treatment to have some merit for situations in which larvae are concentrated. Such concentrations of some *Chrysops* species occur at lake or stream margins during certain seasons of the year. It therefore appeared worthwhile to determine the toxicity of several insecticides against one of these species for possible subsequent use in field experiments.

Larvae of *Chrysops flavida* var. *reicherti* Fairchild were recovered from water-saturated soil on the shore of Alligator Lake, Mississippi during Feburary and March 1957. They were separated from the soil in large Berlesse funnels warmed by heat lamps and then placed in beakers containing water and retained in a constant-temperature cabinet at 20° C. until needed. Usually at least 48 hours was allowed for acclimatization to the higher temperature.

MATERIALS AND METHODS.—The test technique was similar to that used in screening mosquito larvicides (Gjullin & Peters 1952). Fifty milliliters of distilled water was measured in a 250-ml. glass, and a solution or emulsion freshly prepared in the laboratory from the technical chemical was added to make the desired concentration. The contents were agitated by swirling the glass prior to putting 20 larvae in the medium. The test vessel was then placed in a cabinet at 23.5° C., a temperature at which the larvae become moderately active. At least two replicates of each insecticide at each concentration were completed. All the larvae were of the same size, 12 to 14 mm. in length.

Mortality of larvae was recorded at 24, 48, and 72 hours. The 48-hour data was the most consistent and therefore was selected for presentation for the purposes of this paper. Any larva not capable of movement when probed with a dissection needle was considered dead.

Twenty insecticides of the phosphorus, chlorinated hydrocarbon, and thiocyanate groups were evaluated. They were variously formulated depending upon their toxicity to *Chrysops* larvae in relation to their water solubility. Several excellent solvents, such as xylene, were highly toxic to larvae and thus their use was precluded. Acetone and methyl alcohol in the amounts required did not appear to affect the test results.

RESULTS.—The results are presented in table 1. The data indicate a higher tolerance to insecticides for this organism, than, for example, for mosquito larvae (Gjullin & Peters 1952). In general, the phosphorus insecticides caused higher mortalities than the chlorinated hydrocarbons or thiocyanates at equivalent concentrations. Parathion was the most toxic, providing 100% kill at 5 p.p.m. in both an acetone-water solution and an oil emulsion. Lindane was outstanding among the chlorinated hydrocarbons, giving 95% mortality at 10 p.p.m. in six replicates. It was as effective as any phosphorus compound except parathion.

Dieldrin in an oil emulsion was only moderately toxic; however, its residual toxicity might give it an advantage over more toxic but less stable materials. The work of Hansens (1956) with lindane and dieldrin supports this thought.

Lethane 384® (2-(2-butoxyethoxy)ethyl thiocyanate) and toxaphene consistently caused the least kill of larvae at all concentrations. No mortality was caused by any of the vehicle minus the insecticide, although slight variations occurred with parathion or Chlorthion® (*O*-(3-chloro-4-nitrophenyl)*O*,*O*-dimethyl phosphorothioate) depending upon the solvent in which they were carried.

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[2] In cooperation with the Delta Branch of the Mississippi Agricultural Experiment Station.

Table 1.—Toxicity of various insecticides to Chrysops larvae.[a]

Insecticides	Solvent	Per Cent Mortality in 48 Hours at Indicated P.P.M.						
		1	5	10	25	50	100	200
Organic-phosphorus:								
Phosdrin®[b]	Water	0	—	0	—	0	47	55
Dipterex®[c]	Water	—	10	35	50	40	80	—
Malathion	Acetone	—	—	0	70	85	100	—
Methyl parathion	Acetone	—	75	95	80	100	—	—
Parathion	Acetone	80	100	100	—	—		—
	Aromatic No. 5[d] plus Triton X-100	—	100	100	100	—	—	—
Chlorthion®	Acetone	—	40	60	80	—	—	—
	Methyl alcohol	—	—	—	100	100	—	
Guthion®[e]	Acetone	—	—	—	80	80	80	—
Co-Ral®[f]	Acetone	—	90	90	100	—	—	—
Diazinon®[g]	Acetone	—	0	70	80	—	—	—
	Kerosene plus Triton X-100	—	40	70	100	100	100	
Demeton	Kerosene plus Triton X-100	—	—	—	0	10	50	100
Chlorinated hydrocarbons:								
Lindane	Acetone	30	—	95	—	—	—	—
Chlordane	Kerosene plus Triton X-100	—	—	—	—	—	5	35
Heptachlor	Kerosene plus Triton X-100	—	—	—	—	0	30	50
DDT	Kerosene plus Triton X-100	—	—	—	—	35	57	83
Dieldrin	Aromatic No. 5 plus Triton X-100	—	—	—	—	55	15	55
Aldrin	Aromatic No. 5 plus Triton X-100	—	—	—	—	—	10	20
Toxaphene	Aromatic No. 5 plus Triton X-100	—	—	—	—	—	0	0
Endrin	Methyl alcohol	—	—	—	—	50	70	90
Thiocyanates:								
Lethane 384	Kerosene plus Triton X-100	—	—	—	—	10	0	0
Thanite®[h]	Kerosene plus Triton X-100	—	—	—	—	—	10	50

[a] Check mortality nil.
[b] 1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical.
[c] dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate.
[d] Distilled petroleum hydrocarbon.
[e] *O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate.
[f] *O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate.
[g] *O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate.
[h] Isobornyl thiocyanoacetate—82%.

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A Colorimetric Method for the Determination of Co-Ral in Animal Tissues[1]

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ABSTRACT

The use of Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) as a topical spray for the control of cattle grubs has created a demand for a method for determining residues of this pesticide in animal products. A colorimetric method has been developed, and clean-up procedures have been worked out for determining Co-Ral in fat and other tissues. The method has a sensitivity of 0.1 p.p.m. on a 50-gram sample or 0.2 p.p.m. on a 25-gram sample.

Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) (called Bayer 21/199) appears to be very effective for the control of cattle grubs (*Hypoderma lineatum* (De Vill.) and *Hypoderma bovis* (L.)) (Brundrette *et al.* 1957). Its use on livestock has brought about the need for an analytical method to determine residues in animal tissues and milk. A colorimetric method has been developed at the U. S. Department of Agriculture laboratory at Kerrville, Texas, which is sensitive and specific. It is based upon the following chemical reactions:

(1) The Co-Ral is heated in dilute sodium carbonate solution to open up the lactone ring of the umbelliferone radical; and (2) the phenolic compound produced is reacted with 4-aminoantipyrene in an alkaline oxidizing

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medium to give a red compound. The use of 4-aminoantipyrene as a reagent for phenols is well known (Emerson 1943; Gottlieb & Marsh 1946), but its use for determining coumarin-type compounds has not been described.

Gottlieb & Marsh (1946) have stated that the reaction of phenols with 4-aminoantipyrene must be carried out at a definite pH, but this pH is sufficiently alkaline to open the lactone ring in coumarin-type compounds. Compounds of this class other than Co-Ral that have been found to react are coumarin, 4-methylumbelliferone, and 3-chloro-4-methylumbelliferone. With the last-mentioned compound the color is not stable. No other coumarin compounds were available for testing, but apparently the reaction is a general one.

The 3-chloro-4-methylumbelliferone is formed by the hydrolysis of Co-Ral and could be present in animal tissues. It would cause some interference in the analysis, but the chromatographic column used in the clean-up procedure removes it. Unfortunately since this breakdown product does not form a stable color with 4-aminoantipyrene it cannot be determined by the described method.

REAGENTS AND APPARATUS:

Sodium carbonate solution (0.25% made up weekly).
Sodium carbonate solution (0.025% made up daily).
4-Aminoantipyrene solution (2.0% made up daily).
Potassium ferricyanide solution (8.0%).
Purified nitromethane (technical nitromethane triple-distilled and washed with 5-per cent sodium carbonate shortly before using).
Nitromethane-pyridine. 10:1 mixture of purified nitromethane and reagent-grade pyridine (made up only a short time before using).
Standard Co-Ral. Purified sample obtained from Chemagro Corporation, P. O. Box 4913, Kansas City, Mo.
Spectrophotometer, Beckman Model B with test-tube attachment and 19×105 mm. round cuvettes.

PREPARATION OF STANDARD CURVE.—Prepare a standard solution containing 10 μg. of Co-Ral per ml. in acetone. To seven 30×150 mm. test tubes add 0, 1, 2, 3, 4, 5, and 6 ml. of this solution. Place a glass bead in each tube, immerse in a boiling-water bath, shake until ebullition starts, and leave in the bath until the acetone evaporates. Add to each tube 1 ml. of acetone and 2 ml. of 0.25% sodium carbonate. Heat the tubes in a boiling-water bath for 10 minutes. Remove from the bath, cool, and add 0.5 ml. of 4-aminoantipyrene solution. Swirl the tubes and add 5 ml. of 0.025% sodium carbonate, followed by 0.35 ml. of potassium ferricyanide. Mix and let stand for 10 minutes. Add 8 ml. of nitromethane-pyridine solvent and swirl for 1 minute. Pour the mixture into the cuvettes and centrifuge for 10 minutes at 2,000 r.p.m. Thirty minutes after addition of the potassium ferricyanide take the optical-density readings of the nitromethane layer, without removing the upper aqueous layer, at 495 millimicrons, and plot the standard curve from these readings (fig. 1).

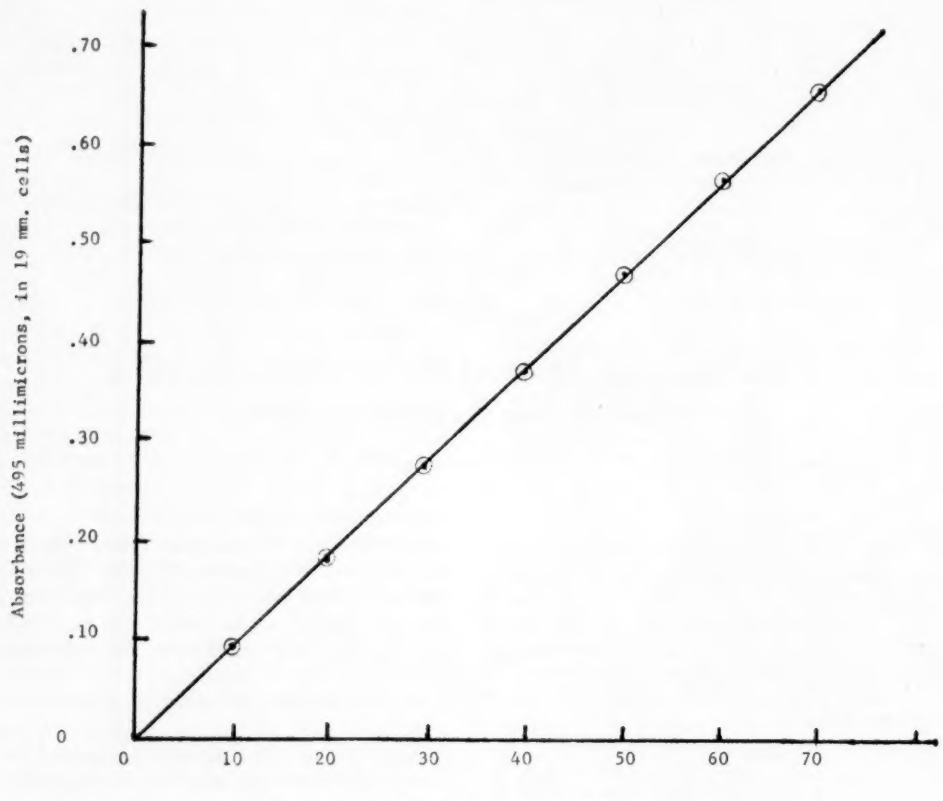

FIG. 1.—Co-Ral standard curve.

Greater sensitivity may be obtained by using smaller volumes of the nitromethane-pyridine solvent. Eight milliliters is the smallest volume that can be read in the cuvettes described above, but by using glass beads or a marble to plug the cuvettes the volume may be reduced to 5 ml.

ANALYSIS OF ANIMAL TISSUES.—*Beef fat.*—Extract a 25-gram sample of fat with benzene. Distill off the benzene to a volume of 50 ml. and then evaporate the remainder, using a vacuum to remove the last traces. Dissolve the fat in 200 ml. of n-hexane in a separatory funnel and extract four times with 50-ml. portions of nitromethane. Drain the nitromethane extracts into another separatory funnel containing 200 ml. of n-hexane so that each extract is successively washed with hexane. Combine the nitromethane extracts and distill off the solvent to a volume of 10 ml. Add 50 ml. of n-hexane and evaporate to dryness; then add 30 ml. more and again evaporate to dryness to make sure the nitromethane is completely removed.

Prepare a column of Florisil (40/60 mesh) 18×150 mm. and wash with 50 ml. of n-hexane. Put the residue on the column using about 50 ml. of n-hexane. Wash the column with 200 ml. of n-hexane containing 2% of acetone. Change the receiver and elute the column with 250 ml. of n-hexane containing 3% of acetone. Distill off the solvent from this fraction to 10 ml., and then transfer the residue to a 25×150 mm. test tube, using acetone to make the transfer. Evaporate to dryness in a boiling-water bath and develop the color as described for the preparation of the standard curve.

Since there are wide variations in different lots of Florisil, the chromatographic column should be calibrated to determine where the Co-Ral comes off. After calibration it may be necessary to change volumes of n-hexane or concentrations of acetone.

Beef Liver and Muscle Tissues.—Chop a 25-gram sample into small pieces and mix in a blender with 200 ml. of absolute alcohol. Transfer the mixture to a 200-ml. centrifuge bottle and spin at 3,000 r.p.m. for 10 minutes. Decant the alcohol into a 300-ml. flask and concentrate to 50 ml. by distillation. Transfer the solid residue from the centrifuge bottle back to the blender, add 15 grams of anhydrous sodium sulfate, and blend with 150 ml. of n-hexane. Filter the mixture through a folded filter, wash the blender, and filter with 75 ml. of n-hexane.

Table 1.—Recovery of Co-Ral from beef tissues.

MICROGRAMS ADDED	WEIGHT OF TISSUE (GRAMS)	PER CENT RECOVERY		
		Fat	Liver	Muscle
10	25	87	—	—
20	25	—	—	92
30	25	94	90	84
50	25	94	84	94
70	25	98	86	—
80	25	99	—	—
30	50	—	—	80
70	50	—	—	80

Transfer the concentrated alcohol extract to a separatory funnel and dilute with 50 ml. of water and 100 ml. of saturated salt solution. Add 10 ml. of 5% sulfuric acid and extract with n-hexane. For the first two extractions use the n-hexane extract from the filtration above. Then extract twice more with 75-ml. portions of fresh solvent. Combine the n-hexane extracts and wash with 75 ml. of saturated salt solution. Drain the salt water off as completely as possible, and then extract the n-hexane solution four times with 50-ml. portions of nitromethane. Distill off the nitromethane to 10 ml., evaporate to dryness, and put through the Florisil column in the same manner as for the fat analysis.

RECOVERY DATA.—When different amounts of Co-Ral were added to beef fat, muscle, and liver tissues, recoveries of 80% to 99% were obtained by the procedure described. These results are shown in table 1. The blanks were equivalent to 2.9 micrograms on a 25-gram sample of fat and 3.9 on a 50-gram sample. The blanks on 25-gram samples of liver and muscle were equivalent to 1.5 and 0.7 micrograms, respectively. The method has a sensitivity of 0.1 p.p.m. based on a 50-gram sample and 0.2 p.p.m. based on a 25-gram sample.

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ERRATUM

In volume 50, page 132 (April 1957) footnote 5, the chemical name for Bayer 23129 should have been:

S-2-(ethylthio)ethyl *O,O*-dimethyl phosphorodithioate

The Effect of Drying and Various Diluents on Spermatozoa of the Honey Bee (*Apis mellifera* L.)[1]

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ABSTRACT

Honey bee semen air dried 3, 6, and 9 minutes contained respectively 60%, 10%, and less than 1% motile spermatozoa after being stimulated with saline solution. Spermatozoa in semen diluted with several different saline solutions and stored at 35° C. in fine glass capillary tubes remained alive for 5 to 10 days. The stored semen was adversely affected by drying and bacterial growth.

Undiluted semen kept at 35° C. in flame-sealed capillary tubes contained many actively motile spermatozoa after 22 days and a few motile spermatozoa after 34 days.

A saline solution was found necessary to restore activity to the spermatozoa which, shortly after ejaculation, assumed a passive condition indistinguishable from death.

Spermatozoa of the honey bee (*Apis mellifera* L.) are remarkable for their longevity within the spermatheca of the queen. Queen bees often live 3 years or longer and continue to produce eggs fertilized by spermatozoa received soon after emergence.

Methods for preserving and extending bee semen (spermatozoa and accompanying fluids) would be of value for improving the results of artificial (instrumental) insemination of queen bees and also for importing valuable genetic stock by the shipment of semen. Importation of adult bees would be easier but is prohibited by Federal quarantine. Useful diluents may probably be developed by empirical testing of likely solutions under different conditions of storage. However, additional basic information is needed on the factors responsible for spermatozoan longevity within the spermatheca of the queen.

The possibility of using diluents for honey bee semen has been considered for many years. One of the earliest to use a diluent was McLain (1886), who added a drop of glycerine and warm rain water to semen before he attempted artificial insemination of queen bees. Since then many different fluids have been used, most of them different types of physiological saline solutions (Howard & France 1917, Fiat 1923, Morison & Blackshaw 1951, Smirnov 1953).

Dissecting fluids have been used commonly in studies of spermatozoa of insects other than honey bees. Koltzoff (1908) found spermatozoa of a rhinoceros beetle still alive after a week in a 2% "tripotassium citrate" solution. He attributed their death to bacterial growth in the solution. In contrast, Nonidez (1920) reported that a 1% potassium citrate solution rapidly injured spermatozoa of *Drosophila* and they lived only 2 to 3 minutes. He also found other fluids harmful, including tap and distilled water, 0.5% saline, cold-blooded Ringer's saline, and 0.1% sodium hydroxide. Payne (1934) used Belar's and Locke's media in studying spermatozoa of the boxelder bug, *Leptocoris trivittatus* (Say). Locke's medium abnormally activitated the spermatozoa and its use was discontinued in favor of Belar's medium adjusted to isotonicity.

Despite the knowledge that honey bee spermatozoa live for years within the spermatheca of the queen, little progress has been made in demonstrating longevity of spermatozoa *in vitro*. The record appears to be held by Rösch, who kept spermatozoa alive and apparently normal for 24 hours or more (Nolan 1929). Most other workers have reported figures for spermatozoan longevity similar to those of Kurennoi (1954), who observed that spermatozoa lived from 10 minutes to 3 hours at temperatures varying from 8° to 46° C.

The work reported here was done at Davis, California, in 1951–52 with the objectives of evaluating the effects of air drying on honey bee semen and finding a practical diluent for it. In order to attain these objectives it was first necessary to develop techniques for collecting, storing, and testing bee semen.

MATERIALS AND METHODS.—Semen was obtained readily from mature drone bees by causing them to evert the penis and ejaculate when pressure was applied to the thorax and abdomen. The semen was collected from the tip of the everted penis. The drones used were usually caged at emergence and held in strong queenless colonies until they became sexually mature at 7 to 10 days of age. Sometimes free-flying drones were caught at hive entrances.

Drying experiments were conducted by exposing measured amounts of semen, containing active spermatozoa, to the air at room temperature and relative humidity (22.8°–28.9° C., 49 to 53% R.H.) for 3-, 6-, and 9-minute periods. Samples of semen (0.366 mm.^{3}) were examined for motility, exposed to air for the required period, covered with an equal volume of Baker's saline (see formula D), and sealed as a hanging drop. Each sample was then observed at 3-minute intervals for 15 minutes and rated each time for its percentage motility. Longevity of the spermatozoa was not rated. Baker's saline was used to revive the dried samples because preliminary observations indicated that it acted as a strong stimulant on bee spermatozoa.

During initial diluent tests, semen was observed for 2 hours in hanging drops of distilled water, bee blood, and Baker's saline. The effect on spermatozoa of a simple saline, Solution A (see formulas), with four levels of added dextrose was also observed in hanging drops for periods up to 6 hours. Each observation required the semen sample from an individual drone. Several methods of handling the samples were then tested to find one that would achieve adequate mixing of the semen and diluent and allow periodic examination of samples of semen from only one drone.

The technique evolved made use of fine hand-drawn

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capillary tubes for holding the diluted semen. Equal volumes (0.366 mm.^{3}) of semen and diluent were placed on a coverslip and sealed as a hanging drop for 10 minutes. The mixture was then drawn into the capillary using the Mackensen (1954) syringe adapted to attach to the base of the tubing from which the capillary was drawn. The filled capillary was broken from its base, plugged at each end with soft paraffin, and placed on a slide warmer at 35° C. Each evening the semen in the filled capillary was sampled by cutting a 2- to 3-mm. piece from the tube and placing it in a hanging drop of Baker's saline. The condition of each sample (color, consistency) was noted and an estimate was made of the spermatozoan motility immediately and approximately 12 hours later.

When different materials were tested as plugs for closing the capillary tubes, flame sealing was rejected as dangerous to the enclosed semen. It was finally tested on three fine capillaries partially filled with undiluted semen. One was opened 15 days, the second 22 days, and the third 34 days after being sealed.

Materials tested as diluents were basically saline solutions modified in accordance with reports of success or failure in maintaining viability of spermatozoa of various animals, including honey bees. Glycine was included because it noticeably increased the longevity of spermatozoa of sea urchins (Giese & Wells 1952) and also of birds (F. W. Lorenz, personal communication). Dextrose is a common ingredient in saline solutions and has been used successfully in diluents for spermatozoa of chickens (Koch & Robillard 1945) and of rabbits (Emmens 1947).

Formulas for the saline diluents used in the experiments were:

A with dextrose		*B with dextrose*		*C with glycine*	
NaCl	700 mg.	NaCl	700 mg.	Glycine	225 mg.
KCl	25 mg.	KCl	25 mg.	NaCl	800 mg.
Dextrose 50, 100, 150, 250	mg.	$CaCl_2$	20 mg.	KCl	20 mg.
Water	100 ml.	$MgCl_2$	10 mg.	$CaCl_2$	20 mg.
		Dextrose	100 mg.	$MgCl_2$	10 mg.
		Water	100 ml.	Water	100 ml.

D (Baker's)[a]		*E*	
Dextrose	3000 mg.	Potassium citrate	2000 mg.
$Na_2HPO_4 \cdot 12H_2O$	600 mg.	Water	98 ml.
NaCl	200 mg.		
KH_2PO_4	10 mg.		
Water	100 ml.		

[a] Baker (1932).

Water redistilled over glass was used in all solutions.

RESULTS.—The drops of semen subjected to 3, 6, and 9 minutes of air drying became scalelike with a hard outer layer. Samples exposed for 3 minutes contained an average of 60% motile spermatozoa, which moved actively through the fluid after Baker's saline (D) was added for stimulation. Semen samples exposed for 6 minutes contained an average of approximately 10% motile spermatozoa. Many were coiled or partially coiled, an indication of injury. Nine minutes of air drying appeared to be near maximum for survival of spermatozoa. Some samples exposed this long regained less than 1% motile spermatozoa, while others failed to show any sign of life. In many samples exposed to the air, groups of spermatozoa became fastened together so that they appeared to be one large organism, five or more times broader than a typical spermatozoon. The groups superficially resembled nematodes or giant spermatozoa as they moved through the saline solution. They were identified by fixing and staining some of the preparations.

The diluents tested initially (water, bee blood, and Baker's saline) were observed over such a short period that their full effect on spermatozoa was not determined. Distilled water seemed to reduce the motility of some samples but the spermatozoa regained at least 90% motility when Baker's saline was added 2 hours later. Spermatozoa in semen diluted with bee blood retained an average motility of about 15% after 90 minutes. Undiluted check samples and those diluted with Baker's saline appeared nonmotile in 60 minutes.

Results of tests of saline solution A with four levels of added dextrose are shown in table 1.

When the diluted semen was stored in capillaries, observations were as follows:

1. Saline A with 100 mg. dextrose—slight motility after 8 days.
2. Saline B with dextrose—slight motility after 6 days.
3. Saline C with glycine—fair motility after 10 days.
4. Potassium citrate, solution E—moderate motility after 5 days. All samples contained tightly coiled spermatozoa, a sign of injury.
5. Check samples, undiluted—slight motility after 10 days.

When the first flame-sealed capillary was opened after 15 days, some of the spermatozoa became motile immediately within the tube and in the hanging drop of Baker's saline (D) where some of the semen projected. After about 8 hours, spermatozoa had moved out into the fluid in large numbers and were very active. When the second flame-sealed capillary was opened after 22 days, little motility was evident at first. After 6 hours, movement was visible within the capillary and spermatozoa had migrated completely around the edge of the hanging drop. The third capillary was opened 34 days after sealing. The semen appeared to be dry and somewhat yellow, with no visible spermatozoan movement. Eight hours later bacteria were evident in the hanging drop and no spermatozoa had migrated into the fluid. Twelve to fifteen spermatozoa at each end of the tube were slowly undulating. No other movement could be seen.

DISCUSSION.—The methods of testing diluents evolved gradually as information was developed about the reactions of the spermatozoa. At first, continuous motility of the spermatozoa was regarded as a measure of their viability, as it is for spermatozoa of many mammals. This criterion cannot be used for studies of honey bee semen because the spermatozoa, after a short period of motility following ejaculation, become inactive. This

Table 1.—Motility of spermatozoa in semen diluted with saline solution A.

DILUENT A	PER CENT MOTILITY	
	Immediately	After 6 Hours[a]
Saline+ 50 mg. dextrose	100	25
Saline+100 mg. dextrose	100	50
Saline+150 mg. dextrose	100	1
Saline+250 mg. dextrose	100	1
Undiluted check	100	75[b]

[a] A second application of the respective diluents was made just prior to estimating the motility after 6 hours.

[b] Saline with 100 mg. dextrose was added before estimating motility.

condition cannot be distinguished from death unless the spermatozoa are again stimulated to activity. The longer they have been inactive the more difficult they are to stimulate, or the slower they are to react. A differential stain would be of value in distinguishing live from dead honey bee spermatozoa. The stain used by Shaffer & Almquist (1949) for differentiating live and dead bull spermatozoa was tested with negative results during this study. In all observations, motile spermatozoa were considered to be only alive, not necessarily capable of fertilization. According to Stephen Taber, III, of the Bee Culture Laboratory, Baton Rouge, Louisiana (personal correspondence), spermatozoa which were motile when thawed after storage in dry ice accomplished virtually no successful fertilizations.

The reports of the very short life of honey bee spermatozoa have probably been based on the duration of motility after ejaculation. Fiat (1923) and Smirnov (1953) indicated that water was toxic to spermatozoa and killed them very rapidly. In the present work, water shortened the initial period of activity but did not kill the spermatozoa within a 2-hour period. In studying the vitality of spermatozoa at different temperatures, Kurennoi (1954) found average longevities as low as 4 minutes at 10° C. At 34° C. he found the average survival time was only 57 minutes. This conclusion compares closely with the 60-minute period of motility of spermatozoa in undiluted semen observed in an early experiment in this investigation. It seems certain that Fiat, Smirnov, and Kurennoi confused the cessation of activity of the spermatozoa with their actual death.

Early in the course of the work it was found that inactive spermatozoa were stimulated into moving by the heat and/or light from the microscope light when samples were being examined. Because of the stimulation, subsequent samples were maintained on a slide warmer at 35° C., the temperature of the honey bee brood nest.

The use of capillaries for storing the semen, and the greatly increased period of observation, introduced desiccation and bacterial growth as complications in testing the diluents. The paraffin plugs, although they were the best of the materials tried, did not consistently prevent the gradual drying of the semen in the capillary. Each time a piece of the tube was removed, the remaining portion to be sealed had semen at the very end. The paraffin often did not make proper contact with the capillary wall and allowed the semen to become dry. The adverse effect this had on the experiments was demonstrated by an accident involving the loss of identity of several capillaries. They were left unsampled for 8 days and the enclosed spermatozoa were still moderately active when stimulated 13 days after being placed in the tubes. In comparison, spermatozoa in tubes sampled daily could be stimulated to motility for an average of only 7.8 days.

Although drying was a serious problem in testing diluents, it does not appear to be an important factor in artificial insemination. The semen is rarely exposed to the air for more than a few seconds during an insemination, far less than the 3-minute experimental exposure that caused only moderate reduction in motility of spermatozoa.

Bacterial growth was not a problem when observations were made over short periods. When the observation was extended to more than 3 days, large numbers of bacteria, especially motile, rod-shaped forms, were seen in the hanging drops. The effect of the bacteria on the activity and longevity of the spermatozoa could not be determined, but they undoubtedly changed the composition of the media, thereby complicating the evaluation of the different fluids. They were additionally undesirable because they did not occur in all samples. The inclusion of an antibiotic or similar substance in the diluent, as in bull semen diluents, would allow better evaluation of fluids being tested. Such a material should also aid in the maintenance of spermatozoa over long periods and reduce loss of queens which results from insemination with semen contaminated by drone fecal matter or other infectious material.

The results of these investigations show that honey bee spermatozoa have an amazing vitality which should make it possible to develop a diluent for practical use. To be of value in artificial insemination, a diluent needs only to be relatively nontoxic for a period of 6 hours. After this period the spermatozoa placed within a queen's reproductive tract have either migrated to the spermatheca or have been ejected or absorbed (Bishop 1920). On reaching the spermatheca the spermatozoa are probably free of the diluent and any transitory detrimental effect it may have exerted. The three saline solutions tested appear to be safe enough for use in artificial insemination.

A diluent for extending and preserving bee semen for shipment between continents needs to be nontoxic over a much longer period, probably 1 to 2 weeks. For this purpose, a fluid with a bacteria inhibitor will probably be required.

Diluents may serve an additional function in bee breeding by increasing the volume of semen. Woyke (1956) and others have found that large volumes of semen are necessary to achieve near-normal insemination artificially. Although this relationship probably results from the tremendous number of spermatozoa contained in the larger volumes, it could also be a function of the volume of fluid introduced. If so, a diluent could be used with advantage to increase the volume of semen in single-drone inseminations used for genetic studies.

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DDT Deposits on Grapes as Affected by Growth and Weathering[1]

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ABSTRACT

Disappearance of surface deposits of DDT on grapes was determined for sprays applied for control of the grape berry moth, *Paralobesia viteana* (Clem.). Methods of sampling grapes for these determinations are described. The results of analyses from 7 years are reported for samples collected in the period beginning immediately after application of final spray and extending into the harvest period. A deposit of less than 7 p.p.m. was found at harvest when only two first-brood sprays are applied. While residues at harvest were below the tolerance for three sprays when no spreader-sticker was used, such a schedule gives poor insect control. With a spreader-sticker in 6 out of 7 years, the deposit of DDT was 17.0 to 22.8 p.p.m. immediately after the final spray application and 6.1 to 12 p.p.m. at the beginning of harvest. The contribution of each application to the deposit from a three-spray schedule was determined. Losses in amounts of deposits are given separately for growth and weathering. The former caused decreases averaging 27.7% in 4 years and weathering 37.2%. The influence of adhesive and of fungicides on the initial deposits and subsequent losses are reported.

An important phase of studies of the grape berry moth, *Paralobesia viteana* (Clem.), the major grape pest of the Chautauqua Grape Belt of New York, has been a consideration of various pesticide residue problems encountered in its control by chemical means. Initially interest centered in surface residues present at harvest on fruit treated with lead or calcium arsenate (Taschenberg 1948). Similar data were taken on DDT, which superseded the arsenicals in berry moth control. In addition, studies were also undertaken to determine the DDT residues at different steps in processing fresh grapes into juice and jam (Taschenberg & Avens 1949). The present paper reports on the influence of growth and weathering on the rate of reduction of DDT spray deposits after the completion of various spray programs.

In New York two DDT sprays are customarily applied to control the first brood of the grape berry moth and one for the second brood (Taschenberg 1948, 1953). The first treatment is applied immediately after blossoming, the second 8 to 12 days later, and the third usually during the first half of August. The final application is made about 6 weeks prior to harvest. Experience has shown a requisite of control to be that fruit be completely covered with the insecticide. Although control is usually improved by the inclusion of a spreader-sticker such materials increase the persistence of the deposits and result in higher harvest residues (Taschenberg *et al.* 1950).

PROCEDURES.—All the residue investigations were made on the variety Concord. The vineyard selected in 1949 had vine growth below average and was used only one season. From 1951 through 1957 treatments were made in a planting where vine growth showed a decided increase in 1951, 1952, and 1953, and thereafter vigor was well above average.

General.—Since the work was carried on independently of insect control studies, one row of 70 grape vines constituted a plot. Spray-drift complications were essentially eliminated through use of a hooded boom. All applications were made with a hydraulic sprayer operating at 500 pounds pressure employing a boom equipped with 14 nozzles. Spray was applied at a 225-gallon rate for the first and second treatments and 300 gallons for the final one. Prior to 1952 DDT was combined with bordeaux mixture, 4-4-100 for the first and second applications and 2-4-100 for the third application. An emulsible oil was added at the rate of 3 pints per 100 gallons. From 1952 to 1957 inclusive, the ingredients of the spray mixtures were: DDT, ferbam, and 8 ounces of an emulsible oil in the first spray; DDT combined with low soluble copper, lime and 1 quart of emulsible oil in the second and third sprays.

DDT residues were removed by transferring a weighed sample to a half-gallon fruit jar, adding 500 cc. of benzene, and mechanically rotating for about 15 minutes.

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[2] The authors acknowledge the assistance of H. J. Crowe in collection of grape samples for this investigation.

All samples were weighed and stripped within 24 hours after collection. Determinations of DDT deposits were made according to the method described by Schechter *et al.* (1945) as modified by Downing & Norton (1951). An untreated or check sample was included with each series of treated samples. Analyses of these checks gave blank readings which ranged from 0.1 to 0.9 p.p.m. in 1951; 0.6 to 1.0 in 1952; 0.3 to 1.0 in 1956 and 0.0 to 0.3 in 1957. Blank values were deducted from those of the treated samples. Grapes treated with DDD were handled, stripped, and analyzed in the same manner as fruit treated with DDT.

Sampling.—Prior to 1951 samples for analysis consisted of berries taken from 12 clusters, picked at random on 4 vines. From 10 to 20 berries were selected from each cluster (Taschenberg & Avens 1949). The weight of such lots ranged from 250 to 400 grams. Experience indicated there might be an advantage in selecting a larger and more representative sample. Hence, in 1951 and continuing through 1957 samples were chosen as follows: Berries were taken from three selected clusters on each of 20 vines. These bunches were not removed but five berries of average size were selected at random from each cluster and, to avoid handling, were cut off with shears, along with a short length of pedicel, and allowed to fall into a paraffin-coated paper pint container. Thus, 300 berries comprised the standard sample. Samples were taken from a new series of clusters at each sampling. Since the vines were trained to the umbrella kniffen system, berries were picked from clusters located in the zone between the top and bottom wire of the trellis. Clusters were avoided where they were either densely protected by foliage or were entirely exposed. Samples were taken over a period of 8 to 10 weeks.

Studies were made in 1956 to obtain further information on the number of berries needed per sample. Analyses were made on lots of 300, 600, and 900 berries. All were taken from different sections of 20 vines, each in the same row. The collection of samples was done according to the procedure described above, except that in 600- and 900-berry lots, 10 and 15 berries, respectively, were picked from a cluster. Also, deposits were determined on 300-berry samples taken from two or more treated replicates. All samples were collected by the same person.

Table 1 shows that the results were similar in all sample sizes. This fact applied to both light and heavy residues. Hence, one may conclude that a 300-berry sample is adequate for residue determinations of this nature. One objection to the larger samples, especially 900 berries, is mechanical, for as the fruit approaches maturity, the danger of cracking or crushing the berries during stripping is increased as the number of berries above 300 is increased. Additional data on 300-berry samples are given in table 2. On duplicate samples taken from the same row little difference in amounts of residue was found. The greater variation was found on samples taken from different rows treated with the same schedule. This situation is attributed more to vine variation than to faulty sampling.

During 1957 the problem of sampling received further attention. The main objective of the work was to compare three methods of sampling. All samples were taken in duplicate lots of 300 berries each. The sampling was limited to 20 consecutive vines in one row. In the first procedure 5 berries were picked per cluster from 3 clusters on each of the 20 vines, the standard procedure described earlier. Before selecting samples by the other procedures, the 20 vines were divided into 5 groups of 4 vines each. From each group of 4 vines 15 clusters were selected but not removed, and 5 berries were taken from every cluster. The selection of clusters was subjected to the same restrictions used when taking samples by the first procedure. For sample collection by the third procedure the entire crop was harvested on each group of four vines. The berries were cut from the stem. As this was being done the berries were assembled in a layer one berry deep on a table $2\frac{1}{2}$ feet by 12 feet. The layer was divided into 10 equal parts and 30 berries were picked at random near the center of each section. Throughout these operations an effort was made to keep handling to a minimum. The results of residue determinations on samples collected by the various methods are given in table 3.

In general rather small differences were found in residues present on lots of berries collected by the same sampling method. Greater variation was found on sam-

Table 1.—Comparison of DDT deposits in p.p.m. on different size samples of grape berries treated with two and three sprays, 1956.

Number Berries	2 DDT Sprays		3 DDT Sprays	
	Weight Sample (Gm.)	Residue (p.p.m.) 10/25	Weight Sample (Gm.)	Residue (p.p.m.) 10/11
300	935	4.3	1,052	7.0
300	873	3.8	1,035	7.0
300	—	—	1,007	7.6
Average		4.1		7.2
600	1,748	4.2	2,024	7.2
600	1,911	4.4	1,951	7.1
600	—	—	1,955	7.0
Average		4.3		7.1
900	2,564	4.3	2,974	5.9
900	2,674	3.7	2,970	6.8
900	—	—	3,000	8.2
Average		4.0		7.0

Table 2.—Comparison of amounts of DDT deposit in p.p.m. on samples of 300 berries each from the same and from different rows, 1956.

Number Applications				DDT Residue (p.p.m.)				
1	2	3	Row	8/22	9/5	9/19	10/3	10/17
7/4	7/14		1	4.7	4.5	3.8	2.9	2.8
7/4	7/14		1	4.6	4.8	3.7	3.0	2.9
Average				4.6	4.6	3.7	2.9	2.9
7/4	7/14		2	5.9	4.8	4.9	4.2	3.9
7/4	7/14		2	5.0	5.9	5.6	4.4	4.1
Average				5.4	5.3	5.2	4.3	4.0
7/4	7/14	8/22	3	16.9	11.0	6.8	6.1	6.3
7/4	7/14	8/22	4	14.1	8.6	6.3	6.0	5.4
7/4	7/14	8/22	5	18.0	10.3	6.4	5.6	5.3
Average				16.3	10.0	6.5	5.9	5.7

ples picked according to the various procedures. Of significance is the agreement in deposits for samples taken by two of the procedures; namely, the first employed from 1951 to 1957, with 15 berries per vine picked from a total of 20 vines, and the third procedure which prescribed the selection of aliquots of fruit from a pool containing all the berries from groups of four vines. The conclusion is that the former method, followed throughout most of the investigation, provides representative lots of fruit for residue analysis. In addition this procedure is practical from the standpoint of time required to collect samples. Close agreement in amounts of residue on duplicate samples from each group of four vines is an indication of the uniform coverage given by the spraying equipment used to treat the grapes.

Growth.—One of the chief factors accounting for the apparent reduction of pesticide residues on grapes is growth. Thus since spraying is terminated before the fruit has completed its development some "reduction" in deposit at harvest is a reflection of increased fruit weight. During the course of the present investigation measurements were made several seasons not only of the weight increases between the end of spraying and harvest but also of the surface area increases. Measurements were recorded at biweekly intervals for 7 or more 300-berry samples and used in connection with the DDT residue determinations. Surface area data were obtained by measurements in a suitable graduated cylinder of the displacement of water by the berries.[3]

Table 4 records increases in weight and surface area of Concord grapes at biweekly intervals from time of final application until after beginning of harvest. For 5 years the weight increases varied from 25% to 32% and averaged 27.7%. For 4 years the surface area increases ranged from 9% to 19% and averaged 15.6%. In general, maximum growth was attained within 4 to 6 weeks after the last spray application.

Of significance in the spray residue problem on grapes is the effect of surface area on the amount of deposit. Per given weight the area is much greater for grapes than for most tree fruits in the period 6 to 8 weeks prior to harvest. At this time the total surface area of 1 pound grapes, 210 berries, is about five times the area of the same weight of apples of $2\frac{1}{2}$ inches in diameter, table 5. From the last application to harvest the area of a 1-pound sample changed relatively little.

Table 3.—Residues of DDT on samples of 300 berries collected by various sampling procedures, 1957.

Sampling Procedure		DDT Residue p.p.m., 9/24[a] by Replicates 1	2	3	4	5	Average
1. 20 vines, 300 berries							
3 clusters per vine	A	9.2					
5 berries per cluster	B	9.1					
	Average	9.2					
2. 4 vines, 300 berries							
15 clusters per vine	A	7.0	6.6	5.2	5.7	7.9	
5 berries per cluster	B	7.1	6.8	5.7	6.3	7.7	
	Average	7.1	6.7	5.5	6.0	7.8	6.6
3. 4 vines, 300 berries selected							
All clusters per vine	A	9.6	9.0	9.1	8.9	10.2	
All berries per cluster	B	7.7	9.3	8.6	9.4	10.1	
	Average	8.7	9.2	8.9	9.2	10.2	9.2

[a] Sprays applied 6/21, 7/2, and 8/13.

Table 4.—Increase in weight and areas of Concord grape berries for the 8-week period beginning immediately after last spray and extending into harvest season, 1951, '52, '53, '55, '56, and '57.

Days After Final Application	Average Weight of Berry (Gm.)	Per Cent of Maximum Weight Attained	Average Area of Berry[a] (Cm^2.)	Per Cent of Maximum Area Attained
1951	*Final application 8/13*		*Harvest 9/26*	
0	1.98	73.4	7.46	81.4
14	2.11	77.7	7.50	81.9
28	2.39	85.3	8.47	92.5
42	2.70	100.0	9.16	100.0
56	2.54	94.3	8.79	96.0
1952	*Final application 8/11*		*Harvest 9/18*	
0	1.98	68.2	7.57	81.0
14	2.24	82.7	8.05	86.2
28	2.59	89.0	8.77	93.9
42	2.91	100.0	9.34	100.0
56	2.90	99.4	9.32	99.7
1953	*Final application 8/19*		*Harvest 9/28*	
0	2.29	71.0	8.24	91.5
14	2.52	82.4	8.60	95.5
28	2.78	100.0	9.00	100.0
42	2.78	100.0	9.00	100.0
56	2.68	99.0	8.90	98.9
1955	*Final application 8/15*		*Harvest 9/19*	
0	1.91	70.6	—	—
14	2.40	88.2	—	—
28	2.69	98.5	—	—
42	2.72	100.0	—	—
56	2.69	98.5	—	—
1956	*Final application 8/22*		*Harvest 10/5*	
0	2.40	75.0	8.52	83.8
14	2.64	82.7	9.05	88.9
28	2.98	93.4	9.65	94.9
42	3.10	97.2	9.87	97.0
56	3.19	100.0	10.17	100.0
1957	*Final application 8/13*		*Harvest 19/23*	
0	2.55	75.9	—	—
14	2.93	87.2	—	—
28	3.18	94.6	—	—
42	3.26	100.0	—	—
56	3.20	95.2	—	—

[a] Measurements on 2,100 or more berries.

Weathering.—Other factors responsible for reduction of surface residues may be classified broadly under weathering. Reduction from these causes is actual. In the case of DDT weathering, losses may be due to chemical decomposition and to attrition. Both are affected by high temperature, high humidity, sunlight, rainfall, and wind.

These effects on DDT residues have been studied by various workers under both field and laboratory conditions. According to Gunther (1945), Sweetman (1945), Gunther & Tow (1946), Chisholm & Koblitsky (1947), and Burgess & Sweetman (1949), high temperatures reduce the DDT residue and humidity increases the rate of

[3] The equation used to calculate surface area is as follows:

$$A = \sqrt[3]{36 \times V^2 \times \pi} \times B.$$

Where A is the total area of sample in square centimeters, V is the average number of cubic centimeters of water displaced by one berry, π is the value 3.1416 and B is the number of berries per sample. In this calculation the berries are assumed to be spheres.

Table 5.—Comparison of surface area of 1-pound samples of grapes, cherries and apples.

Fruit	Number per Pound	Total Surface Area (Sq. In.)	Total Surface Area (Sq. Cm.)
Grapes (at time of last spray)[a]	210	259	1,669
Grapes (at harvest)[a]	162	231	1,490
Sour cherries (medium to small)[b]	209	333	2,148
Apples (2½ inch, diameter)[b]	3	50–60	322–387

[a] An average of the samples on grapes for six seasons.
[b] Information on sour cherries and apples taken from publication by Chapman & Pearce (1935).

such losses. Fleck (1944) claims volatilization occurs too slowly to account for much loss of the chemical. The deposits of DDT are affected by sunlight including the ultraviolet light (Gunther 1945, Gahan *et al.* 1945, Gunther *et al.* 1946, Lindquist *et al.* 1946, Wichmann *et al.* 1946, Chisholm & Koblitsky 1947, Fleck 1949, and Hopkins *et al.* 1952).

Wind causes a reduction in DDT dust deposits soon after application but thereafter such loss occurs at a slow rate (Hopkins *et al.* 1952).

Rainfall affects spray and dust deposits by mechanical means, described by the often used term, erosion. Observations and studies on the reduction of DDT residue by precipitation have been published by Fennah (1945), Gunther *et al.* (1946), Hadaway & Barlow (1949), Ginsburg *et al.* (1950), Kirk (1951), Sloan *et al.* (1951), and Hopkins *et al.* (1952). These reports deal with losses in amount of residues from fruit and foliage. Rainfall totaling less than 1 inch and occurring within less than 24 hours after application of DDT removed most of the deposit from foliage (Hadaway & Barlow 1949). A similar observation was recorded with overhead irrigation when 0.5 inch of water was applied within 30 minutes after application of a spray of DDT wettable powder (Kirk 1951). By allowing a lapse of 25 hours or more between time of treatment and application of water the decrease in original deposit was found to be less. Observations supporting the relationship of time of rainfall to loss of DDT was recorded on lettuce by Sloan *et al.* (1951) and on forage crops by Hopkins *et al.* (1952).

In the course of these investigations on grape residues, weathering was considered in its broadest meaning but only rainfall was measured. The records of rainfall for each season are presented in the tables showing deposit data.

RESULTS AND DISCUSSION.—Changes in deposits are shown graphically in figure 1 from a 3-spray schedule of DDT and from individual treatments of this schedule beginning 5 days after the third application and continuing for a period of 8 weeks. Deposits from sprays applied for each brood of the grape berry moth are presented in table 6. From a spray applied immediately after bloom approximately 1 p.p.m. of DDT was found 56 days later whereas a deposit of 4.5 p.p.m. was present 40 days following the application of one spray 10 days after fruit set, the latter being the second spray for the first brood. It is apparent that the first spray applied after bloom contributes little to the deposit found at harvest. A single spray, the second of a 3-spray schedule, and a schedule of two first brood sprays gave about the same result. Harvest residue from a third spray alone approached the residue from a full 3-spray schedule. Hence, most of the harvest residues was derived from the third spray. The loss of DDT from individual early-season applications was small after the time of third application. This loss is shown by the slopes of the curves in figure 1. Evidence showing the persistence of DDT deposits on grapes is given in table 6. At the end of 90- to 100-day intervals, between the completion of the second spray ap-

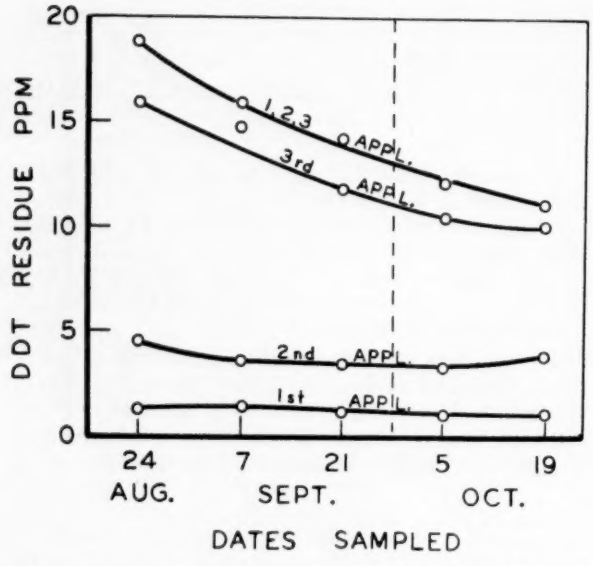

FIG. 1.—Showing decrease in DDT deposits in p.p.m. on Concord grapes treated with a 3-spray schedule and the individual applications of this schedule, 1953, Applied: 1st-6/29; 2nd-7/6; 3rd-8/19. Beginning of harvest indicated by broken line.

Table 6.—Comparison of DDT deposits[a] in p.p.m. on grapes treated with separate schedules, two sprays for first brood; and a single spray for second brood.

Year	Dates of Application[b] 1	2	3	Date of Sampling and DDT Deposits (p.p.m.)					Harvest Began
				First brood sprays					
1952	6/25	7/5	—	8/11	8/25	9/8	9/22	10/5	9/18
				5.2	5.1	3.8	3.2	3.8	
1955	6/21	7/2	—	8/15	8/29	9/12	9/26	10/10	9/19
				6.3	5.0	4.3	3.8	3.1	
1956	7/4	7/14	—	8/22	9/5	9/19	10/3	10/17	10/5
				5.7	4.8	3.8	3.8	3.3	
1957	6/21	7/2	—	8/13	8/27	9/10	9/24	10/8	9/23
				4.4	3.9	2.9	2.8	2.2	
				Second brood sprays					
1951	—	—	8/13	8/13	8/27	9/10	9/24	10/8	9/26
				21.2	14.9	11.9	7.4	8.0	
1952	—	—	8/11	8/11	8/25	9/8	9/22	10/5	9/18
				16.1	12.1	8.5	7.5	6.8	
1953	—	—	8/19	8/24	9/7	9/21	10/5	10/19	9/28
				15.9	14.9	11.8	10.4	10.0	
1956	—	—	8/22	8/24	9/5	9/19	10/3	10/17	10/5
				11.8	5.8	5.9	5.2	3.9	

[a] Deposit determinations for the period beginning after second brood application and extending into harvest.
[b] First spray immediately after bloom.

Table 7.—Reduction of DDT and DDD deposits on grapes following three or four spray treatments, 1949, 1951, and 1952.

MATERIALS (LB./100 GAL.) AND RAINFALL (IN.)	DATES TREATED				DDT AND DDD DEPOSITS (P.P.M.)								
					1949[a]—Harvest date 9/15								
	6/18	*6/28*	*7/27*	*8/10*	*7/27*	*8/10*	*8/17*	*8/24*	*8/31*	*9/7*	*9/14*	*9/21*	*9/28*
DDT (50%) 1.5	+	+	+		21.0	17.3	16.7	12.5	9.9	10.6	8.4	7.4	6.1
DDT (50%) 1.5	+	+	+	+	—	29.5	22.0	21.0	18.9	14.5	13.6	11.1	10.0
DDD (50%) 2.0	+	+	+		22.1	19.4	21.0	14.9	13.1	12.2	9.7	9.7	
DDD (50%) 2.0	+	+	+	+	—	33.1	32.7	23.2	21.4	19.3	15.1	13.3	
Rainfall accumulated					0.00	0.20	0.30	0.94	2.43	2.99	3.74	5.12	6.41
					1951[a]—Harvest date 9/26								
	6/26	*7/5*	*8/13*	*8/27*	*8/13*	*8/27*	*9/3*	*9/10*	*9/17*	*9/24*	*10/1*	*10/8*	
DDT (50%) 1.5	+	+	+		20.4	13.6	11.7	10.4	9.9	8.3	8.8	7.5	
DDT (50%) 1.5	+	+	+	+	—	33.7	26.0	23.0	21.3	20.4	17.1	14.1	
Rainfall accumulated					0.00	0.62	1.45	1.65	2.52	2.70	3.34	4.96	
					1952[b]—Harvest date 9/18								
	6/25	*7/5*	*8/11*		*8/11*	*8/18*	*8/25*	*9/1*	*9/8*	*9/15*	*9/22*	*9/29*	*10/5*
DDT (50%) 1.5	+	+	+		22.8	19.3	17.1	16.6	13.8	12.5	11.4	11.5	10.4
Rainfall accumulated					0.00	1.47	3.64	3.96	5.79	8.64	10.10	10.51	11.58

[a] Bordeaux mixture 4-4-100, 4-4-100 and 2-4-100 was included in first, second, third, and fourth application and 3 pts. of emulsible oil.
[b] Ferbam, 1.5 lb. and emulsible oil, 8 oz., was included in first application and low soluble copper, 2 lb.+lime, 4 lb. and 2 pts. of emusible oil in the second and third application.

plication and the final sampling date, the deposits ranged from 2.2 to 3.8 p.p.m.

Deposits found at weekly intervals on fruit treated with three or four DDT sprays are given in table 7. With insecticides which persist as do DDT and DDD there appears to be no advantage in sampling at weekly intervals. Therefore, after 1952, samples for residue analysis were taken every 2 weeks. Although DDD was used at a higher rate than DDT the initial deposit and rate of loss are similar. Three sprays of DDT and DDD gave harvest residues in excess of 7 p.p.m. In 1949 a fourth spray of DDT increased the amount of deposit by 70% over that remaining from a third spray applied 13 days earlier. More than double the DDT deposit was found following the application of a fourth spray in 1951.

Although not shown in table 7 data are available for 1951 and 1952 which permit the separation of the amount of reduction in deposit due to weathering and growth.

In 1951 the harvest residue from three sprays amounted to 41% of the original deposit, a loss of 59% on a weight basis. Of this decrease 14% was caused by growth and 45% by weathering. Since the amount of precipitation was "normal" for the period of sampling, rainfall is assumed to be only partly responsible for the reduction owing to weathering. At harvest in 1952 the deposit was reduced 23% by weathering and 22% by growth when rainfall was well above the average for the period between last spray and beginning of harvest. In this connection the physical nature of the coverage and/or deposit from spray mixtures warrants some consideration. Along with the insecticide, bordeaux mixture and emulsible oil were used in 1949 and 1951. Ferbam was included in the first spray and low soluble copper and lime and oil in the later sprays in 1952. Sprays of bordeaux mixture, DDT, and oil give somewhat uneven and blotchy coverage of grape berries. As the deposit aged it tended to crack and eventually flake. Thus, the loss owing to weathering is likely to be greater for such sprays than for those containing DDT, low soluble copper, lime and emulsible oil which gave a smooth and more uniform coverage.

From 1952 to 1957 inclusive, deposits were determined from a program of three sprays of DDT containing an emulsible oil as the spreader-sticker and a fungicide. Only a part of the data obtained during this 6-year period is given in table 8. Represented are data showing losses in amounts of deposits to be expected in an average year as well as extremes, and larger than usual loss owing to either weathering or growth. The object of this study was to determine the relative decrease in deposits owing to weathering and growth. Samples were collected at biweekly intervals for an 8-week period beginning immediately after the final spray treatment except in 1953 when the first samples were taken 5 days after the last spray. However, in all years the final sample was picked after harvest was underway. For 3 out of 4 years the initial deposits were similar ranging from 17.0 to 18.9 p.p.m. A very heavy original deposit, 30.6 p.p.m. was found in 1955. This fact may be accounted for in part by greater exposure of clusters because of lighter-than-usual vine growth brought on by the lack of rainfall in June and July. The interval between the last spray and beginning of harvest was from 6 to 7 weeks. At the latter time the residues were in excess of 7 p.p.m. every year except in 1956. An explanation for this lower-than-usual harvest residue is given later when the effect of weathering on deposits is discussed.

Initial deposits of similar amounts were found for two formulations of wettable DDT powder applied in 1957. However, the deposit from the 25% formulation weathered somewhat more rapidly than the deposit from the 50% formulation.

The effect of weathering on deposits tended to follow a rather definite pattern, shown by data given in table 8. The reduction in deposit owing to weathering 6 weeks

Table 8.—Comparison of actual and apparent losses in DDT deposits at bi-weekly intervals from final application until after harvest, 1953, 1955, 1956, and 1957.

Date Sampled	DDT Deposit: By Weight P.p.m.	Grams	Reduction (%)[a]	Per 100-Berry Sample: Micrograms	Reduction (%)[b]	Reduction Due to Growth (%)	Rainfall Accumulated (In.)
DDT, 50%, w.p. 1.5 lb./100 gallons[c]							
1953 Final application 8/19—Harvest date 9/28							
8/24	18.9	230	0	4,340	0	0	0.00
9/7	15.5	250	18	3,875	11	8	1.68
9/21	14.1	269	25	3,797	13	15	4.63
10/5	12.0	263	37	3,160	27	13	4.63
10/19	11.0	262	42	2,886	34	12	5.14
1955 Final application 8/15—Harvest date 9/19							
8/15	30.6	197	0	6,028	0	0	0.00
8/29	20.1	253	34	5,085	16	22	0.51
9/12	18.0	268	41	4,824	20	27	2.61
9/26	14.0	273	54	3,817	37	28	4.20
10/10	13.2	269	57	3,551	41	27	7.63
1956 Final application 8/22—Harvest date 10/5							
8/22	17.0	252	0	4,292	0	0	0.00
9/5	11.0	272	35	2,995	30	7	3.63
9/19	6.8	304	60	2,068	52	17	5.33
10/3	6.1	300	64	1,833	57	17	6.42
10/17	6.2	319	64	1,979	54	21	7.03
1957 Final application 8/13—Harvest date 9/23							
8/13	17.4	255	0	4,444	0	0	0.00
8/27	13.6	291	22	3,958	11	12	0.08
9/10	11.6	328	33	3,802	15	22	1.72
9/24	8.9	359	49	3,194	28	29	6.91
10/8	7.8	329	55	2,564	42	22	6.93
DDT, 25%, w.p. 3.0 lb./100 gallons[cd]							
1957 Final application 8/13—Harvest date 9/23							
8/13	18.4	268	0	4,925	00	0	0.00
8/27	12.4	303	33	3,763	24	12	0.08
9/10	8.2	327	56	2,685	46	19	1.72
9/24	7.5	349	59	2,618	47	23	6.91
10/8	7.3	308	60	2,249	54	14	6.93

[a] Apparent loss.
[b] Actual loss.
[c] Spreader-sticker, emulsible oil; 1 part by volume of B1956 and 9 parts of Sovaspray Oil No. 3.
[d] Commercial formulation, Black leaf 253.

after the final spray, about harvest, ranged from 27% to 57% and averaged 37.2% for 4 years. In 1956 reduction caused by weathering was marked, exceeding the highest for the other years by 20%. This loss appeared to be associated with rainfall. Important in this connection was the amount of precipitation, 3.63 inches. Of this amount two-thirds fell within three rather short periods, 0.64 inches in 2 hours, 0.86 inches in 7 hours, and 0.94 inches in 6 hours. Similar amounts of precipitation were recorded for biweekly periods in other years but the effect on deposits was less evident. As an example consider the interval between the third and fourth sampling period in 1957. For this interval the total precipitation was 5.19 inches, which included rainfall of 0.71 inches in 4 hours, 0.85 inches in 5 hours, and 1.73 inches in 17 hours.

Reduction in deposits owing to growth in experiments conducted in 1953, 1955, 1956, and 1957 are shown in table 8. The apparent decrease in deposit caused by increase in weight is a function of the weight of fruit and the per cent increase in weight. This growth reduction in deposit cannot be expressed simply as the difference between reduction in deposits based on weight and on sample. In the present paper this value is calculated according to the following formula:

$$\frac{B-A}{B} \times 100 = \% \text{ reduction due to growth}$$

A = Average wt. of 100 berries in initial sample.
B = Average wt. of 100 berries at date of sampling.

Within the 6-week period prior to harvest the apparent loss in deposit ranged from 13% to 29% and averaged 21.7% for 4 years. Generally, the rate of decrease was small, with a tendency for it to level off within 4 to 6 weeks after final spray. The patterns of loss in deposits owing to growth in 1955 and 1956 varied greatly from those of other years. During 1955 a larger than usual reduction in deposit was caused by growth within the 2 weeks after the last spray, the decrease being 22%. An irregular increase in weight of fruit occurred under these conditions. A drought period of more than 2 months' duration was followed by a wet period in which precipitation amounted to 5.14 inches for 2 weeks prior to application of the final spray. Reduction caused by growth in 1956 was considerably less than the reduction caused by this factor in other years, amounting to 7% at harvest. Undoubtedly the extreme climatic conditions, heavy rainfall, and low temperatures of that year limited the effect of growth. Weathering decreased the deposits by 52% within 4 weeks after final spray.

For three seasons an emulsible oil and spreader-sticker C[4] were compared as supplements for the DDT spray on grapes. The amount of oil per 100 gallons was 1 quart and the concentration of spreader-sticker C for two seasons

[4] Spreader-sticker, E. I. Dupont Company.

Table 9.—Comparison of actual and apparent losses in DDT as influenced by spreader-stickers from final application until after harvest, 1954, 1955, and 1957.

Date Sampled	DDT Deposit: By Weight P.p.m.	Grams	Reduction (%)[a]	Per 100-Berry Sample: Micrograms	Reduction (%)[b]	Reduction Due to Growth (%)	Rainfall Accumulated (In.)
DDT, 50%, w.p. 1.5 lb./100 gallons							
No Spreader-sticker							
1954 Final application 8/23—Harvest date 9/23							
8/23	10.0	205	0	2,160	0	0	0.00
9/6	6.7	238	33	1,665	22	14	1.00
9/20	4.1	270	59	1,167	46	24	2.04
10/4	2.9	300	71	883	49	32	3.68
1957 Final application 8/13—Harvest date 9/23							
8/13	6.8	251	0	1,709	0	0	0.00
8/27	4.4	291	35	1,283	25	16	0.08
9/10	2.2	333	68	733	57	16	1.72
9/24	1.6	375	77	600	65	27	6.91
10/8	1.6	307	77	492	71	11	6.93
Spreader-sticker A[c]*, 3 oz./100 gallons*							
1954 Final application 8/23—Harvest date 9/23							
8/23	16.1	220	0	3,542	0	0	0.00
9/6	12.6	247	22	3,112	12	11	1.00
9/20	8.0	275	50	2,200	38	20	2.04
10/4	5.5	295	66	1,622	54	26	3.68
1955 Final application 8/15—Harvest date 9/19							
8/15	23.6	198	0	4,681	0	0	0.00
8/29	16.1	230	32	3,707	21	14	0.51
9/12	13.1	254	45	3,327	29	22	2.16
9/26	11.0	254	53	2,799	40	26	4.20
10/10	10.0	263	58	2,635	44	24	7.63
Emulsible Oil[d]*, 16 oz./100 gallons*							
1954 Final application 8/23—Harvest date 9/23							
8/23	18.1	216	0	3,909	0	0	0.00
9/6	13.9	249	23	3,456	12	13	1.00
9/20	9.5	285	48	2,704	31	24	2.04
10/4	9.5	273	48	2,595	34	30	3.68
1955 Final application 8/15—Harvest date 9/19							
8/15	27.7	197	0	5,466	0	0	0.00
8/29	19.9	243	29	4,831	12	19	0.51
9/12	15.0	276	46	4,144	24	29	2.16
9/26	13.5	267	51	3,604	34	26	4.20
10/10	11.5	260	59	2,990	45	24	7.63

[a] Apparent loss.
[b] Actual loss.
[c] Dustic, Niagara Chemical Div., Food Machinery and Chemical Corp.
[d] Spreader-sticker, emulsible oil; 1 part by volume B1956 and 9 parts Sovaspray Oil No. 3.

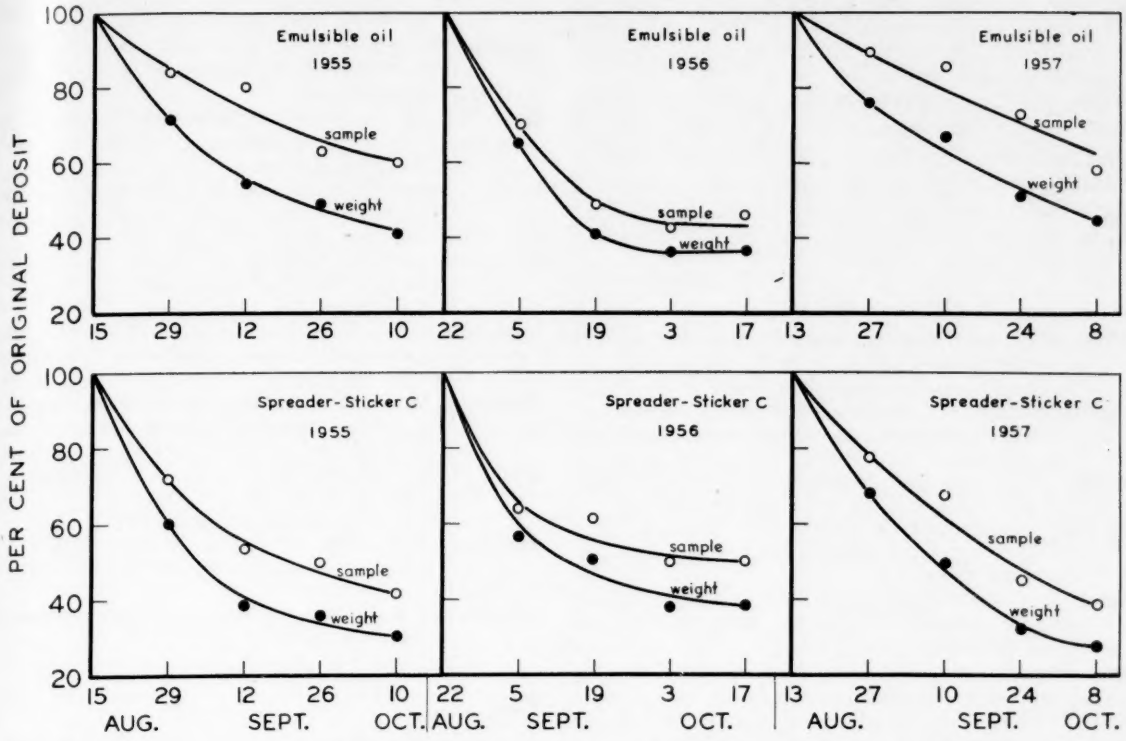

FIG. 2.—Disappearance curves for DDT deposits when oil or spreader-sticker C was used. Sample curves show actual rate of loss caused by weathering and weight curves show rate of loss owing to combined effect of weathering and growth.

was 8 ounces and in the third season the concentration was lowered to 6 ounces in an effort to reduce the loss in deposit caused by weathering. Data from the study on these supplements are presented graphically in figure 2. Shown are the decreases in deposits caused by weathering and weathering plus growth. On a sample basis large differences in decrease of deposits for emulsible oil and spreader-sticker C are seen in 1955 and 1957. Such differences did not exist in 1956. However, in 1956 a rapid rate of decrease in deposit was observed with both supplements, undoubtedly caused by the heavy rainfall and high winds. During the period of 8 weeks in which samples were collected the total rainfall was about the same in each of the 3 years but there are large differences in amounts of precipitation in the 2-week intervals between each sampling. Of particular significance was amount of rainfall in the 2-week interval following collection of initial samples. This feature was discussed in detail earlier in this section.

Additional data on disappearance of deposits are given in table 9 for schedules of three DDT applications when a spreader-sticker was present or omitted. These results may be compared with those for the standard supplement for grape sprays, 1 quart of emulsible oil, shown in table 8. By omitting the supplement the initial deposit was much lighter than that found for sprays containing a spreader-sticker, 8.4 p.p.m. (average of 2 years) compared with 21 p.p.m. (an average for 4 years). At harvest the deposit ranged from1.6 to 4.1 p.p.m. on fruit treated with sprays to which no supplement was added. Weathering of such deposits exceeded that found for other spray mixtures. The initial deposits with spreader-sticker A and with 1 pint of miscible oil show slight differences, the former giving a slightly smaller amount, also, the deposit with spreader-sticker A appears to weather more rapidly than the deposit when a miscible oil was used. With 1 pint and 1 quart of emulsible oil the deposits immediately after last spray were similar and reductions in these deposits caused by weathering were about the same. Visible coverage of fruit was somewhat superior with the higher concentration of oil.

CONCLUSIONS.—The DDT-spreader-sticker schedule of two first-brood treatments and one second brood treatment applied for grape berry moth control gave deposits of from 17 to 23 p.p.m. immediately after the final application and a harvest deposit usually in excess of the tolerance of 7 p.p.m. Much of this deposit was contributed by the final spray applied for the control of the second brood. With two first-brood applications the deposit at harvest was below the tolerance, being from 2.5 to 4 p.p.m. An interval of about 80 days is represented. By omitting a spreader-sticker in a schedule of three post-blossom sprays, the residue was less than 7 p.p.m. at harvest.

Weathering caused a loss in the initial deposit ranging between 27% and 57% in the period of from 6 to 8 weeks following the application of the last spray to harvest. In the absence of high winds the grape foliage tends to protect the fruit from the direct erosive effect of rainfall. The fungicide and adhesive used also affect the weathering of deposits. Lowest losses occurred with DDT combined with a low soluble copper fungicide plus lime and an emulsible oil.

Since the final spray treatment was discontinued before grape berries have completed development, some of

the "loss" in deposit was attributable to growth reflected by increase in weight. In these studies this factor has accounted for from 13% to 29% in the apparent reduction of the deposit.

The combined effect of weathering and growth caused a reduction in DDT deposits at harvest which ranged from 37% to 64% over 4 years, and averaged 51%. While weathering accounted for the actual decrease in deposit, growth was also an important factor in affecting the final amount found since the official spray residue tolerances are based on weight.

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Control of Pear Borer and American Plum Borer in Apple Trees[1]

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ABSTRACT

In the spring of 1956 a serious infestation of American plum borer, *Euzophera semifuneralis* (Wlk.), and pear borer, *Thamnosphecia pyri* (Harr.), was found in Summer Rambo (9-year-old) and Lodi (15-year-old) apple trees. Sprays of dormant oil 2 qts.+Gamtox® (10% gamma isomer of BHC w.p.) or Isotox® (20% gamma isomer of BHC from lindane)=to 1 lb. BHC 10% gamma, gave only 60% control, when applied with an orchard broom. Subsequently (1957), sprays of 15% parathion at 2 lbs. w.p. or 1 pt. of 47.5% Paraflow® (water emulsion containing 4 lbs. parathion per gallon) emulsion applied at 400 p.s.i. with shade-tree guns gave perfect control of borers, without reinfestation for a 2-year period.

Early in July 1956 the attention of the junior author was directed to a serious attack of borers on the trunks of 9-year-old Summer Rambo and 15-year-old Lodi apple trees. The attack had already caused the death of an appreciable number of trees and many others showed an unhealthy appearance. Two types of larvae and pupal cases were associated with the injured portions of the trees. The predominant species was a white larva with a light brown head, which constructs a cocoon of silk and frass similar to that produced by the peach tree borer, *Sanninoidea exitiosa* (Say). This larva was determined to be the pear borer, *Thamnosphecia pyri* (Harr.). The sec-

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ondary species had a dark lavender-colored larva which spins a loose silken cocoon under the bark and was identified as the American plum borer, *Euzophera semifuneralis* (Wlk.).

The larvae worked under the loose bark and fed down to the cambium layer, resulting in an oozing of sap and producing characteristic "weepy spots" readily observed on the trunks of the trees. The distinct difference in the two larvae and their pupal cases made field identification and separation of the species positive.

The original search of the literature produced only four references of interest: papers by Forbes (1890), Sanderson (1901), and Blakeslee (1915) reported life-history studies and controls for the American plum borer, while the last by Brooks (1920) covered the life history and control of the pear borer. The only control suggestion worthy of trial at the time of the present research was hand scraping of the bark with consequent destruction of the larvae. However, with the present high cost of labor, this measure could be recommended only as a last resort. A fifth paper by Woodside (1952) was overlooked in the original search, and much valuable information on the action of modern organic insecticides on the pear borer was not found until after the conclusion of the experiment.

During the 55 years since Sanderson (1901) reported on the injury produced by the American plum borer in Delaware orchards, no reports of its presence in this State have been recorded. The discovery of the pear borer was a new record for Delaware.

PROCEDURE.—On July 11, 1956, 60 nine-year-old Summer Rambo trees, showing injury, were tagged for test purposes. These were divided into 15 groups of 4 trees each. On July 20, two trees in each group were scraped and two left unscraped, and the following materials were applied at the rate of 1 pint per tree at 45 to 60 pounds' pressure with a Hudson #115 hand sprayer:

Treatment	*Materials*
1	Orthol D® (97% emulsifiable, Viscosity/100° F., 90 seconds; Gravity A.P.I.°, 34; Unsulfonated residue (A.S.T.M.), 96) oil at 2 qts./100
2	Orthol D 2 qts.+Gamtox® (10% gamma isomer of BHC w.p.)=1 lb. 10% BHC/100
3	Orthol D 2 qts.+Gamtox=2 lbs. 10% BHC/100
4	Orthol D 2 qts.+Gamtox=4 lbs. 10% BHC/100
5	Orthol D 2 qts.+Isotox® (20% gamma isomer of BHC from lindane)=1 lb. 10% BHC/100
6	Orthol D 2 qts.+Isotox=2 lbs. 10% BHC/100
7	Orthol D 2 qts.+Isotox=4 lbs. 10% BHC/100
8	Para-Scalecide® (12.7% paradichlorobenzene) 1–7 in water
9	Para-Scalecide 1–7+Gamtox=1 lb. 10% BHC/100
10	Para-Scalecide 1–7+Gamtox=2 lbs. 10% BHC/100
11	Para-Scalecide 1–7+Gamtox=4 lbs. 10% BHC/100
12	Para-Scalecide 1–7+Isotox=1 lb. 10% BHC/100
13	Para-Scalecide 1–7+Isotox=2 lbs. 10% BHC/100
14	Para-Scalecide 1–7+Isotox=4 lbs. 10% BHC/100
15	Check

Examination of the treated trees on August 10 revealed perfect control where the trees had been scraped, regardless of treatment. Where the sprays had been applied to unscraped portions of the trunk, empty pupal cases were found, indicating some sprays had failed to penetrate sufficiently to kill the larvae. Removal of the bark revealed 2 dead larvae and 1 pupal case of American plum borer and 8 dead larvae, 6 dead pupae, 6 empty pupal cases, and 1 pupa of the pear borer on the 30 unscraped trees. Examination of the portions of the trees above the treated areas revealed 17 American plum borers and 70 pear borers in the untreated wood of the 60 trees.

On the basis of the reported experiment, the grower was advised to spray all his Lodi and Summer Rambo trees in the spring of 1957 with a spray consisting of 2 quarts of dormant oil plus Gamtox or Isotox at the equivalent of 1 lb. of BHC (10% gamma isomer)/100. This spray was applied in March with a five-nozzle orchard broom 400 p.s.i. A check of the orchard by the senior author on May 17 and 21 revealed only about a 60% kill of borers and much rough bark suitable for oviposition. It was obvious that much of the trouble in control resulted from a lack of drive of the spray material as it hit the tree and, as a consequence, the insecticide did not reach the site of borer activity.

Following the authors' directions, the grower constructed two guns of the type used to spray shade trees from old orchard brooms, pipe, and reducing nipples. On completion, the guns had tubes about 6 feet long, reduced to an orifice of $\frac{3}{16}$ inch in diameter. At 400 p.s.i. they were capable of removing all the bark from a tree, when the nozzle was held steady at 3 to 4 feet from the trunk.

On May 24, 1957, four materials were applied with these guns to a portion of the Summer Rambo block at the rate of 3 gallons of spray per tree at 400 p.s.i. from the ground to as high as rough bark occurred. The guns produced a forceful spray, which not only drove the materials under the bark but also ripped most of the loose bark from the trunks of the trees. The insecticides used and results recorded are presented in table 1.

A second check on September 11, 1957, revealed a further reduction in attack on the sprayed trees; however, both the Gamtox- and DDT-treated trees showed a few larvae or pupal cases and activity evident in three of the seven check trees.

Following the experimental application the grower, on his own initiative, sprayed the trunks of all his remaining Summer Rambo and Lodi trees with Paraflow 1 pt./100 gals.

Inspection of trees in 1958 revealed only one or two trees with borers. In the spring of 1959 the author again checked the trees, which in 2 years had accumulated much rough bark, and could find no evidence of borers. The grower then reported three unhealthy trees and stated that he had removed a few borers from the trunks. One of these trees was in the unsprayed check and the

Table 1.—Sprays applied to Summer Rambo apple trees May 24, 1957, and control recorded July 5, 1957.

MATERIALS AND RATES	TREES EXAMINED	LIVING PEAR BORERS	LIVING PLUM BORERS	WEEPY[1] SPOTS	CLEAN
DDT 50%, wp 4 lb./100	19	4	3	14	10
Gamtox 20% 1 qt./100	14	1	1	9	9
Parathion 15%, wp. 2 lb./100	15	0	0	0	15
Paraflow 47.5% 1 pt./100	16	0	0	0	16
DDT one side; water other side[2]		0	0	0	
	7	2	0	4	5
Check	7	5	2	10	1

[1] Weepy spots indicate activity, but no borer was found.

[2] DDT supply was exhausted before both sides had been sprayed, therefore water was used to remove loose bark on opposite side of trees.

others were in a different part of the orchard.

The greater degree of control obtained in this experiment with a single spray and considerably reduced dosage of insecticide, though at a higher rate per tree (3 gallons per tree of 2 lbs. 15% parathion or 1 pt. 47.5% Paraflow/100) compared with the best control obtained by Woodside (1.25 to 2 gallons per tree in 2 sprays of 3 lbs. 25% parathion in 1 year followed by 5 lbs. of 15% parathion the second year) can be attributed only to the difference in the method of application, namely, a shade-tree gun at 400 p.s.i. capable of dislodging the loose bark and forcing the spray material into the cracks and crevices vs. a single-nozzle orchard gun at 125 p.s.i. which wet only the surface.

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Greenbug Control with Systemic Insecticides as Influenced by Fertilizer Applications[1]

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ABSTRACT

Data secured from 1956 to 1959 through greenhouse experiments, showed that greenbug (*Toxoptera graminum* (Rond.)) control with the systemic phosphorus compounds demeton and Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate) used as soil treatments was little or no better when used in combination with nitrogen and phosphorus fertilizers than when used alone. However, slight differences were realized when demeton was used as a seed treatment.

Control methods that have been developed for the greenbug, *Toxoptera graminum* (Rond.), are not always practical. Most of the available insecticides are ineffective as sprays or dusts at temperatures below 50° F., when greenbugs are feeding and reproducing. Systemic insecticides offer a possibility of control of this insect under conditions where conventional insecticides are not effective. Therefore entomological workers have been investigating the feasibility of applying systemic insecticides, at planting time, to protect small-grain plants from greenbug attack. To date a systemic insecticide that will remain effective from planting time in the fall until the following spring, when greenbug damage is likely to occur, has not been found. However, some workers have reported successful aphid control with systemics applied as seed treatments to spring-planted small grains.

Various workers have indicated that soil with different fertilizer or nutrient levels may influence the effective period of a systemic compound. In working with cotton plants Hacskaylo & Ergle (1955) reported that as the nitrogen level in the nutrient solution was decreased, schradan was found to accumulate in the plant in significantly larger quantities. A similar but weaker trend was also noted when the phosphorus level was varied. However, the potassium in the nutrient had little or no effect on schradan absorption. Other observations support the theory that small grains grown in soil with nutrients optimum for healthy, vigorous plants are more capable of withstanding greenbug infestations than are those grown under less favorable conditions. Arant & Jones (1951) and Daniels (1957) showed that greenbugs per plant unit varied inversely with the amount of nitrogen applied.

Greenbug infestations are common on the High Plains of Texas and fertilizer practices vary there. Therefore greenhouse experiments were conducted at the Southwestern Great Plains Field Station at Bushland to determine the effects of fertilizer combinations on greenbug control with the systemic compounds demeton and Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate).

METHODS.—Eight-inch flowerpots were filled with Pullman silty clay-loam soil obtained from land on which wheat had been grown. The treatments were arranged in a randomized block and replicated four times. The fertilizers were applied in bands 1 inch below and ½ inch to the side of the seed. Westar wheat was planted in a row ½ inch deep across each pot. The systemic insecticides, which were used as soil treatments, were applied in the same rows or bands as the fertilizer. For the seed treatments, the insecticide was applied to the seed along with a sticker. The plants were thinned to four per pot and infested with two or three adult greenbugs each 6 days after emergence. Chemically protected plants were reinfested at 3- or 4-day intervals until a greenbug colony developed. Three experiments were conducted, one during the winter of 1956–57, one in 1957–58, and another in 1958–59.

Demeton, the insecticide used in the 1956–57 test, when used as a seed treatment was applied at the rate of 0.5 pounds actual per 100 pounds of seed; when used as a soil treatment it was applied at the rate of 20 pounds per acre. Di-Syston, which was used in both the 1957–58 and 1958–59 tests, was applied only as a soil treatment at the rates of 2 and 10 and 0.5 and 2 pounds per acre, respectively. Demeton was 50% actual toxicant on activated carbon and Di-Syston 2.5% granulated material. Nitrogen was supplied as ammonium sulfate (21% N) and phosphorus as concentrated superphosphate (45% P_2O_5).

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Table 1.—Number of days wheat plants were protected from greenbug infestation and plant survival following demeton treatments and fertilizer applications, Bushland, Texas, 1956–57.

Treatment[a]			Plant Protection	Plant Survival
Demeton	N	P_2O_5	Days	Days
0	0	0	0	25
0	60	60	0	36
0.5 (seed)	0	0	34	57
0.5 (seed)	60	60	52	80
20 (soil)	0	0	129	matured
20 (soil)	60	60	140	matured

[a] Pounds per acre with the exception of the demeton seed treatment which was 0.5 pound per 100 pounds of seed.

RESULTS.—The number of days wheat plants were protected from greenbug infestation and the number of days that the plants survived following the use of demeton and fertilizers are presented in table 1. The demeton seed treatment used in combination with nitrogen and phosphorus gave plant protection for 18 days longer than when no fertilizer was used. Consequently, the fertilized plants survived longer. However, the treatment which received nitrogen and phosphorus but no demeton survived 11 days longer than the treatment that received neither fertilizer nor demeton. This conclusion is indicative that a considerable amount of the reduced greenbug damage may have been associated with plant vigor. The systemic soil treatment used in combination with the fertilizers also gave 11 days more plant protection than the demeton soil treatment without fertilizers. As expected the 20-pound soil treatments protected the plants much longer than the seed treatments, and these plants grew to maturity.

The results of the 1957–58 Di-Syston treatments are shown in table 2. There was little or no difference in plant survival time in the treatments receiving no Di-Syston, which is in contrast to the treatments receiving no demeton and where fertilizers were used at a lower rate. This contrast may have been caused by a difference in greenhouse growing conditions. In the treatments receiving Di-Syston, plants were not completely protected immediately. Time varied from 17 to 27 days from the first infestation until greenbugs disappeared from the plants. There were practically no differences in period of plant protection when Di-Syston was used at the rate of 2 pounds per acre regardless of fertilizer combination. A rate of 10 pounds per acre improved results only slightly, which is in agreement with Reynolds *et al.* (1957), who used Di-Syston as a seed treatment against the spotted alfalfa aphid, *Therioaphis maculata* (Buckton). Plants of both the 2- and 10-pound Di-Syston treatments were sufficiently protected from greenbug infestation to reach maturity.

Results of the 1958–59 test are also presented in table 2. Survival time, in the treatments receiving no Di-Syston, varied from 33 days for the plants receiving no fertilizers to 40 days for the plants receiving both nitrogen and phosphorus. Time varied from 27 to 39 days before plants of the Di-Syston treatments were completely protected. There were slight differences in plant protection time and survival time for the treatments receiving 0.5 pound of Di-Syston per acre. Plants of both 2-pound Di-Syston treatments reached maturity, although plant protection time was less than for the 2-pound rates of the 1957–58 experiment. This was again probably caused by greenhouse growing conditions.

Table 2.—Number of days before wheat plants were protected from greenbugs, days of protection and plant survival following Di-Syston treatments and fertilizer applications, Bushland, Texas, 1957–59.

Treatment[a]			Days Before Complete Plant Protection Began	Days		
Di-Syston	N	P_2O_5		Plant Protection	Total[b]	Plant Survival
			1957–58			
0	0	0	—	0	0	27
0	120	120	—	0	0	28
2	0	0	24	106	130	matured
2	120	120	25	106	131	matured
2	120	80	27	104	131	matured
2	120	0	26	104	130	matured
2	0	120	24	104	128	matured
2	0	80	22	100	122	matured
0	0	120	—	0	0	29
10	0	120	24	120	144	matured
0	120	0	—	0	0	28
10	120	120	17	129	146	matured
			1958–59			
0	0	0	—	0	0	33
0	100	100	—	0	0	40
0.5	0	0	39	55	94	123
0.5	100	100	37	55	92	124
0.5	100	0	36	58	94	129
0.5	0	100	36	59	95	126
0	100	0	—	0	0	39
0	0	100	—	0	0	36
2	0	100	27	89	116	matured
2	100	100	32	87	119	matured

[a] Pounds per acre.
[b] Number of days from plant emergence until permanent greenbug colony developed.

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A Study of the Histological Effects of *Hypoderma lineatum* (De Vill.) on Gullet Tissue Taken from Systemically Treated and Untreated Cattle[1]

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ABSTRACT

An evaluation was made of the histopathological effects of the systemic insecticide, *O*-(3-chloro-4 methylumbelliferone) *O,O*-diethyl phosphorothioate (Co-Ral®; Bayer 21/199), on the gullet tissues of cattle sprayed with a 0.5% concentration and cattle drenched at the rate of 10 mg./kg. body weight for control of the common cattle grub (*Hypoderma lineatum* (De Vill.)). Good control of grubs with the spray and none with the drench resulted. A study of the prepared sections revealed no difference in the actual tissue of the grubs, except in case of the dead grubs. An examination of the gullet connective tissue surrounding the grubs revealed the following: *Tissue from Sprayed Animal:* Tissue and cells appear to be normal, *i.e.* normal numbers of macrophages, lymphocytes, plasma cells, and fiberblasts, and collagenous fibers. The spray apparently had no effect on the connective tissue around the parasites or on the walls of the esophagus. The esophagi from the sprayed animals were normal. *Tissue from Drenched Animal:* Very heavy infiltration of lymphocytes, macrophages, and plasma cells, and fiberblasts, with active inflammation was observed. In the submucosa, loose and reticular connective tissue fibers were normal. Quite a number of plasma cells and macrophages were present. Many polymorphoneuclearcytes were present, indicating active inflammation probably owing to drench. Also noticeable was a mild to acute inflammation of the connective tissue fibers and cells of the lamina propria of the esophagus. The inflammation was doubtless caused by the reaction of the host to one or two foreign agents, namely, the larval stages of *H. lineatum*, and the administration of the systemic insecticide. This probably indicates that the use of Co-Ral as a drench not only failed to give grub control, but was detrimental as well.

Although the common cattle grub, *Hypoderma lineatum* (De Vill.), has been known to man for centuries, only quite recently has much been discovered about its bionomics. In reality, two distinct species of cattle grubs are known, the northern cattle grub or ox warble, *Hypoderma bovis* (L.), and the common cattle grub.

Even though the life histories of these species have now been worked out, certain points of controversy and dispute still remain. However, it is generally agreed that the larvae of *H. lineatum* and *H. bovis* tend to follow entirely different courses in the body of the host. Since this research deals only with *H. lineatum*, the present discussion will pertain entirely to it.

This species enters the skin of the host cattle as young larvae and then probably follows the paths of least resistance through the connective tissue until it reaches the neck, at which point the larvae bore through the neck muscles and finally reach the gullet. According to Hadwen & Bruce (1916) such larvae are usually located in the submucosa of the esophagus, gradually working their way toward its diaphragmatic end. Frequently they may follow the posterior borders of the ribs under the pleura and sometimes may enter the neural canal.

Apparently some of the effects of the larvae on the esophageal tissues, other than their mere physical presence, are caused by the secretion of a substance called a hypodermatotoxin, first reported by Hadwen (1918). This material has since been reported on by a number of workers including Ono (1932), and Haga (1944). Essentially it is a protein substance containing glucose and a number of proteolytic enzymes with an action strongly proteolytic and extremely effective in inhibiting coagulation of the blood.

In addition to the action of the hypodermatotoxin, the digestive enzymes of the larvae would certainly have an effect upon the histological changes observed in the gullet. A quantitative study of these enzymes was made by Simmons (1939), in which he determined that the enzymes lipase, rennin, trypsin, and erepsin were secreted by third instar larvae.

The economic importance of the cattle grub has been stressed many times and it has been reported infesting from 33 to 75% of all cattle in this country, with an annual loss ranging from $50,000 to $120,000,000 (Metcalf, Flint & Metcalf 1951). This fact in itself has been sufficient to stimulate considerable research directed toward finding protective insecticides for cattle attacked by the grub. Of those materials under study a very promising systemic insecticide recently appeared (Brundrett *et al.* 1957). This was Bayer 21/199, which chemically is *O*-(3-chloro-4 methylumbelliferone) *O,O*-diethyl phosphorothioate and is sold under the trade name of Co-Ral®.

This paper presents the results obtained from treating cattle with Co-Ral as a spray and orally as a drench. A comparison is made between the histopathological changes in the gullet tissues surrounding the larvae in treated cattle and those tissues surrounding the larvae of untreated cattle. Although Simmons (1937) made a similar study on *Hypoderma* larvae in esophagi of cattle, no comparison between systemically treated and untreated cattle is available. Since the action of Co-Ral is unknown, whether it affects the grub directly or the esophageal tissues or both, it was thought that a study of this nature might be of value in determining some of these unknown factors.

MATERIALS AND METHODS.—*Field Procedure.*—Nine Hereford calves were purchased for this investigation at public auction at New Orleans, Louisiana, during the first week of June 1958. These animals were transported to the South Mississippi Branch Experiment Station at

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[2] Now with the Agricultural Chemical Division, Swift & Company.

[3] The authors wish to express their appreciation to Dr. J. W. Ward, Department of Anatomy, University of Mississippi Medical Center for the basic histological diagnosis of the tissues reported on in this paper.

McNeill and were placed on an improved summer pasture.

On September 23, 1958, after being weighed on platform scales, the nine animals were divided at random into three groups. One group was given an oral drench with a solution of Co-Ral prepared from a wettable powder containing 25% of the active ingredient at the rate of 10 mg./kg. of body weight. The second group was sprayed with a 0.5% water solution of the 25% Co-Ral, wettable powder. This material was applied with a power hydraulic sprayer at a pressure of approximately 200 to 300 pounds. Each animal was thoroughly saturated with the spray mixture. The last group received no treatment and was turned back into a pasture with the other two groups for 2 weeks.

On October 7, 1958, 15 days after treatment, the nine calves were slaughtered at the Bryan Brothers Packing Plant, West Point, Mississippi. Since cattle grubs are known to be concentrated in the esophagus at this time of the year, the gullets of all nine calves were retained for examination and recovery of the embedded larvae. Two of the gullets were shorter than the others, owing to the fact that parts of them were accidently discarded. This loss was not considered important because the remaining portions of these two gullets, 6 and 12 inches long, were considered sufficient for study purposes. Later in the day all of the larvae extracted from the infested gullets were placed in preservative solutions for later sectioning.

Laboratory Procedure.—A careful examination of the gullets revealed that all except two had one or more larvae present. The larvae, creamy or dull white in color and 8 to 12 mm. long, were located, as expected, in the submucosa or connective tissue between the muscles and epithelium.

The grubs and portions of the connective tissue surrounding them were extracted from the gullet by holding the grubs and the connective tissue up with forceps to stretch them slightly away from the main body of tissue and then cutting around the grub with a small pair of scissors. The connective tissue and whole larvae were then placed in bottles containing Bouin's fixative. After a minimum of 18 to a maximum of 24 hours the larvae and connective tissue were taken from the Bouin's solution and prepared for imbedding. The two methods of imbedding employed were the paraffin and celloidin techniques. Routine steps were followed in staining the sections with Delafield's hematoxylin and eosin and Gomori's trichrome using Harris' hematoxylin.

RESULTS.—*Grub control.*—An examination of table 1 indicates that Co-Ral applied for the control of cattle grubs was effective as an external spray but ineffective as an oral drench. One or more grubs were found in each of seven out of nine gullets examined. The sprayed calves had the fewest grubs with a total of three, two of which were dead. The drenched calves had the most grubs with a total of 77, which were all alive. The untreated calves had a total of 29 living grubs. Two gullets, one from the sprayed group and one from the untreated group had no grubs. An examination of table 1 indicates that effective control of grubs was obtained with the spray but not with the drench.

HISTOLOGICAL RESULTS.—*Tissue from Untreated Animal.*—Connective tissue surrounding parasite: Collagenous fibers make up the predominant connective tissue fiber seen in the preparation. The fibers are heavy, irregularly arranged and quite dense. Interspersed at intervals may be seen a small number of elastic fibers, and also a small amount of adipose tissue. A reticular tissue framework showed well in the Gomori's stained sections. The fiberblast was the most prevalent connective tissue cell in these preparations. They were distributed between the collagenous fibers. Some were arranged in a more or less orderly fashion, while others were distributed at intervals. The next most prevalent type of cell was the lymphocyte. Medium and small lymphocytes were seen in clusters which surrounded the capillaries. Many of the capillaries were engorged with cells of which lymphocytes comprise the majority. This infiltration of lymphocytes prevailed throughout the section. Three other types of connective tissue cells were present also, namely, macrophages, neutrophiles, and plasma cells, either in the clusters of cells or distributed at random throughout the tissue.

Tissues of the esophagus proper were normal.

Tissue from Sprayed Animal.—Tissue surrounding parasite: Tissue cells appeared to be essentially the same as the tissue described as "normal," except that more macrophages and not so many lymphocytes were present. Tissue of the esophagus proper was normal.

Tissue from Drenched Animal.—Connective tissue surrounding parasite: All of these sections showed a very heavy infiltration of lymphocytes, macrophages, neutrophiles, and a slight increase in plasma cells. Lymphocytic infiltration was present throughout the tissue. The majority of the capillaries were engorged with cells, the most of which were lymphocytes. Macrophages were seen in clusters throughout the tissue. The tissue of the layers of the esophagus proper except the submucosa, seemed to be normal. In this layer quite an increase in the number of neutrophiles was noticeable, as well as a slight increase in macrophages and lymphocytes. There was a mild to acute inflammation of the connective tissue fibers and cells of the outer surface of the esophagus and of the connective tissue fibers and cells of the lamina propria of the esophagus.

DISCUSSION.—Observations from the sprayed calves

Table 1.—A comparison of two methods of administration of Co-Ral for the control of cattle grubs.

CALF NO.	WEIGHT (LB.)	LENGTH OF GULLET (IN.)	NO. OF GRUBS		
			Living	Dead	Total
		External spray (0.5%)			
996	515	22	0	1	1
997	480	21	1	1	2
999	510	23	0	0	0
		Total	1	2	3
		Oral drench (10 mg./kg.)			
993	410	22	8	0	8
995	400	24	30	0	30
1000	440	12	39	0	39
		Total	77	0	77
		Untreated			
992	420	6	5	0	5
994	510	22	24	0	24
998	390	23	0	0	0
		Total	29	0	29

tended to indicate that most of the grubs were destroyed in the gullet, since two dead grubs were found in the serosal layer of two of the gullets. As shown by the absence of grubs in one of the untreated calves there is a possibility that one of the animals in the sprayed group had no grubs and the remaining two had a very light infestation. However, since five of the remaining six animals in the other two groups had medium to heavy infestations it seems unlikely that the three sprayed animals would be so lightly infested.

The large number of grubs found in the drenched calves indicated no grub control and that internal absorption of the chemical, unlike external absorption, may be reduced by some internal process in the animals' body. This process may consist of the breaking down of the chemical by the digestive tract secretions.

The lack of noticeable differences in the tissues of the grub itself, except in the dead grubs, may be due to no difference, or to the histological techniques used, which may not have been of a nature to reveal any differences.

Whenever tissues or cells are injured or destroyed, an immediate protective response occurs. This response is called inflammation. The basic characteristic of the immediate inflammatory response is almost always the same, regardless of the site of occurrence. The response is composed of a series of physiological and morphological adjustments, involving chiefly the blood vessels, the fluid, the cellular components of the blood, the cellular components of the surrounding connective tissue, as well as the surrounding connective tissue fibers. The inflammatory response destroys, or attempts to destroy the injurious foreign agent or agents, which happen to be the fly larvae, and perhaps, also the drug that was present in the drench.

The engorged blood vessels, the accumulation of lymphocytes, the increase in macrophages, the density of the connective tissue fibers are characteristic of mild acute inflammation as seen in the tissue of the untreated animal. A normal reaction of the host is to destroy, retard, or surround the parasite.

The histological picture, as seen from studying the tissue surrounding the parasite of the animal that was drenched as well as the tissue of the submucosa of the same animal, is one of a typical inflammation of the surrounding connective tissue of the esophagus and of the connective tissue of the lamina propria of the esophagus. There is no doubt that the inflammation of the surrounding connective tissue was caused by the presence of the parasite. It is presumed that inflammation of the lamina propria was caused by the action of the drug in the drench. This fact would indicate that a drench with the tested chemical not only is a poor grub control agent, but that it may be harmful in lowering the animals' resistance to secondary infections in this location.

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Comparative Value of Some Synthetic Compounds as Synergists with Barthrin in House Fly Sprays

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ABSTRACT

The comparative value of 26 new synthetic compounds and of piperonyl butoxide, sulfoxide, and sesamex as synergists for barthrin was estimated in tests against the house fly, *Musca domestica* L., by the turntable method. The most effective compounds were sesamex, 2-[2-(2-butoxyethoxy)ethoxy]ethyl α-ethylpiperonyl acetal of acetaldehyde and its ethoxy analog, and piperonyl butoxide, which raised the toxicity of the mixtures to about three times that expected.

Previous work with barthrin (6-chloropiperonyl chrysanthemumate), the determination of its relatively high toxicity to house flies, *Musca domestica* L., and the evaluation of its intensity of synergism with piperonyl butoxide (Gersdorff & Piquett 1959a, 1959b), naturally raised questions of its joint action with other synthetic compounds. Therefore, tests were made to evaluate some of them as potential synergists with barthrin in comparison with materials known to be synergists with pyrethrins and allethrin—piperonyl butoxide, sulfoxide, and sesamex.

MATERIALS.—The synthetic compounds tested repre-

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sented several molecular types, but all contained a methylenedioxyphenyl group. They were prepared by methods similar to those previously given for related compounds (Beroza 1956, Barthel & Alexander 1958, Alexander *et al.* 1958, Gertler *et al.* 1959). All but four of the samples were purified. The samples of piperonyl butoxide and sulfoxide were technical products obtainable commercially.

The sample of barthrin was that used in the previous studies by the authors; it was 95.2% pure as determined by saponification. A sample of allethrin analyzing 93.4% by the hydrogenolysis method was included in the tests.

Sprays were prepared in refined kerosene (Deobase) at selected concentrations, five for each of the standards and but one for the mixtures. The latter contained 1 mg. of barthrin plus 10 mg. of adjunct per milliliter. This concentration was selected because it caused about 50% mortality when piperonyl butoxide was the synergist (Gersdorff & Piquett 1959b), and therefore would respond with appreciable mortalities for synergists equal to or approaching piperonyl butoxide in effectiveness and yet permit comparisons with more effective synergists.

KNOCKDOWN AND MORTALITY.—The sprays were applied by the Campbell turntable method to house flies of the National Association of Insecticide and Disinfectant Manufacturers' 1948 strain. The tests were made in two series, each series on the same populations of flies. All sprays in a series were tested simultaneously and only once on each of six populations. Approximately 109 flies were used in each test. The average age of flies in each population was 2 to 3 days. The knockdown and mortality data are summarized in table 1.

EVALUATION OF JOINT ACTION.—Table 1 also shows the barthrin equivalents necessary to assess the relative toxicity of the mixtures. To obtain these equivalents and to estimate their experimental error and so obtain the requirements to demonstrate synergism, the following procedure was used: In each series a weighted regression equation of probit mortality on log concentration was computed for barthrin alone essentially as described by Finney (1952). From this equation the logarithms of the barthrin equivalents were calculated for the individual mortalities obtained with all the sprays causing definite mortalities in the series. From an analysis of variance of these log concentrations, the barthrin equivalent required for a significant difference from the actual barthrin content was calculated. This requirement, the relative standard error of the mean barthrin equivalents, and the equations for both barthrin and allethrin (those for the latter having been determined similarly) are also given in table 1. The equivalents reported are the geometric means of the individual equivalents; equivalents corresponding to the arithmetic mean mortalities may be calculated directly from the equations. The latter equivalents differ only slightly, if at all, from the former.

Since the mixtures contained 100 mg. of barthrin per deciliter, their barthrin equivalents are expressions of relative toxicity in per cent. None of the new synthetic compounds, when tested alone by this method at 2,000 mg. per deciliter (that is, twice the concentration in the mixtures), caused a significant mortality. Therefore, a possible contribution to the expected toxicity of the mixtures would be negligible and so is ignored. The barthrin equivalents divided by 100, then, furnish ratios which, when significantly high, express the intensity of synergism in the mixtures with these compounds.

When tested alone by this method at higher concentrations, piperonyl butoxide evinced toxicity to house flies amounting to 0.0329 that of barthrin (Gersdorff & Piquett 1959b) and sulfoxide a toxicity 0.00751 that of allethrin (Gersdorff *et al.* 1952). From the latter ratio sulfoxide is calculated to be 0.0238 as toxic as barthrin—from 0.315, the mean of three estimations of the toxicity of barthrin relative to allethrin (Gersdorff *et al.* 1959). Therefore, the contribution of these two materials to the toxicity of mixtures containing them should be taken into account and the barthrin equivalents given for their mixtures in table 1 should be divided by the expected barthrin equivalents, 132.9 and 123.8, respectively. These intensity ratios, then, are 2.61 and 1.78. The intensity in the barthrin-piperonyl butoxide mixture was estimated previously and more precisely as 2.79 (Gersdorff & Piquett 1959b). From the toxicity of barthrin relative to allethrin and the intensity of synergism for this mixture, it may be deduced that the mixture should be about as toxic as allethrin. A concentration of 132.9 mg. per deciliter when substituted in the allethrin equation corresponds to a mortality of 48%. That caused by the mixture was actually 45%.

Another check on the consistency of results is permitted by the ratio of toxicity of barthrin to allethrin. As cited above, the mean of three estimations of this ratio is 0.315. The mean of the two estimations possible in this study (calculated from the LC-50's given in table 1) is a little higher, 0.352, but this difference is not too great.

Thus, the hope of finding a synergist more effective with barthrin than piperonyl butoxide was not realized with this group of compounds. The seeming superiority of sesamex was not significant in these tests. This synergist was slightly superior in a 10:1 mixture with pyrethrins and on the borderline of superiority with allethrin (Gersdorff *et al.* 1957), thus suggesting a difference demonstrable only with extensive testing. It may therefore be the better synergist with all esters of chrysanthemumic acid, but its superiority from the standpoint of toxicity is hardly of great importance. To be included with these two as the most effective synergists are the 2-[2-(2-butoxyethoxy)ethoxy]ethyl α-ethylpiperonyl acetal of acetaldehyde and its ethoxy analog.

Six materials were moderately synergistic, with intensities of 2 to 2.5. They were 4-(2-bromo-1-ethoxypropyl)-1,2-methylenedioxybenzene; 4-(2-bromo-1-methoxypropyl)-1,2-methylenedioxybenzene; α-(1-bromoethyl)-6-bromopiperonyl *o*-chlorobenzoate; ethyl 3-(3,4-methylenedioxyphenyl)-2-phenylpropionate; butyl α-ethylpiperonyl acetal of acetaldehyde; and sulfoxide.

Slight synergistic action was indicated for 8 other compounds, and the remaining 11 either were on the borderline of significance or were not synergistic.

Because of the lower knockdown potential of barthrin as compared with pyrethrins and allethrin, it appeared likely that the knockdown value of all these mixtures would be low. Such is the case, the most effective causing considerably less than complete knockdown. Complete knockdown is achieved by similar mixtures with pyre-

Table 1.—Joint toxic action against house flies of mixtures of barthrin with candidate synergists (1 mg. barthrin plus 10 mg. adjunct per ml.).

Adjunct or Standard	Per Cent: Knockdown in 25 Minutes	Per Cent: Mortality in 1 Day[a] (Arithmetic Mean)	Barthrin Equivalent[b] (Geometric Mean) (Mg. per Dl.)
Series 1. Six replicates, 110 flies per test			
Acetaldehyde, α-(1-bromoethyl)-6-bromopiperonyl 2-(2-ethoxyethoxy)ethyl acetal[c]	21.9	3.1	169
Acetic acid, chloro-, piperonyl ester	18.8	2.3	—
p-Anisic acid, piperonyl ester	29.0	4.4	177
Benzene,			
4-bromo-5-(2-bromo-1-propoxypropyl)-1,2-methylenedioxy-	13.0	7.7	180
4-(2-bromo-1-ethoxypropyl)-1,2-methylenedioxy-	24.6	18.1	249
4-(2-bromo-1-methoxypropyl)-1,2-methylenedioxy-	23.1	11.4	220
Benzoic acid, *o*-chloro-, α-(1-bromoethyl)-6-bromopiperonyl ester	23.3	11.2	220
Chrysanthemumic acid, α-(1-bromoethyl)-6-bromopiperonyl ester[c]	21.4	2.7	161
Naphthoic acid,			
6-bromo-α-(1-bromoethyl)piperonyl ester	13.6	1.5	—
piperonyl ester	14.0	1.8	—
Octanamide, *N,N*-diethyl-	17.0	0.9	—
Piperonyl butoxide	64.7	44.7	347
Propane,			
1,2-dibromo-1-(2-bromo-4,5-methylenedioxyphenyl)-	21.3	2.2	—
1,2-dibromo-3-(3,4-methylenedioxyphenyl)-	23.0	1.2	—
Propionic acid,			
6-bromo-α-(1-bromoethyl) piperonyl ester	20.8	3.4	171
3-(3,4-methylenedioxyphenyl)-2-phenyl-, ethyl ester	27.1	14.2	231
Sesamex	31.5	47.9	359
Sulfoxide	27.0	11.8	220
Barthrin,			
666.7 mg. per dl.	99.7	91.7	691
444.4	97.4	69.6	470
296.3	87.5	30.4	286
197.5	53.2	3.9	183
131.7	24.1	1.6	142
Allethrin,			
214.1	100	75.9	—
142.8	100	51.9	—
95.17	100	30.5	—
63.45	99.4	10.8	—
42.30	95.2	3.0	—
Required to demonstrate synergism	—	—	121
Equations and LC-50's for standards:			
Barthrin, $Y = 5.5710X - 9.2960$; 368.3 ± 10.2 mg. per dl.			
Allethrin, $Y = 3.6302X - 2.7310$; 136.5 ± 2.4 mg. per dl.			
Relative standard error of mean barthrin equivalent, 7.59%.			
Series 2. Six replicates, 108 flies per test			
Acetaldehyde,			
2-[2-(2-butoxyethoxy)ethoxy]ethyl α-ethylpiperonyl acetal[c]	35.9	28.5	294
butyl α-ethylpiperonyl acetal	13.3	11.9	212
2-[2-(2-ethoxyethoyx)ethoxy]ethyl α-ethylpiperonyl acetal[c]	38.9	26.3	271
Benzene,			
4-bromo-5-[2-bromo-1-(2-butoxyethoxy)propyl]-1,2-methylenedioxy-	21.2	6.1	188
4-bromo-5-[2-bromo-1-(2-ethoxyethoxy)propyl]-1,2-methylenedioxy-	13.7	4.7	173
4-bromo-5-(2-bromo-1-hexyloxypropyl)-1,2-methylenedioxy-	19.1	1.8	—
4-bromo-5-(2-bromo-1-isobutoxy)propyl-1,2-methylenedioxy-	9.3	2.4	164
4-bromo-5-(2-bromo-1-isopropoxy)propyl-1,2-methylenedioxy-	16.4	3.4	175
Isobutyric acid, 6-bromo-α-(1-bromoethyl)piperonyl ester	14.5	5.7	188
Octanamide,			
N,N-dibutyl-	15.1	1.2	—
N,N-dipropyl-	28.1	2.1	153
Barthrin,			
666.7 mg. per dl.	100	89.2	697
444.4	98.1	63.3	450
296.3	91.3	32.0	304
197.5	44.6	2.9	153
131.7	18.5	0.7	—

[a] Refined kerosene alone caused no mortality.
[b] Barthrin equivalent equals relative toxicity in per cent.
[c] Not purified.

Table 1.—(continued)

ADJUNCT OR STANDARD	PER CENT: Knockdown in 25 Minutes	PER CENT: Mortality in 1 Day[a] (Arithmetic Mean)	BARTHRIN EQUIVALENT[b] (GEOMETRIC MEAN) (MG. PER DL.)
Allethrin,			
214.1	100	78.2	—
142.8	100	58.7	—
95.17	100	31.1	—
63.45	99.9	14.6	—
42.30	98.6	2.4	—
Required to demonstrate synergism	—	—	126
Equations and LC-50's for standards:			
Barthrin, Y=5.4596X-9.1096; 384.1 mg. per dl.			
Allethrin, Y=3.7257X-2.8531; 128.2 mg. per dl.			
Relative standard error of mean barthrin equivalent, 9.35%			

thrins or allethrin at half the concentration when they are applied by the same method.

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The Effect of Soil Treatments of Chlordane or Heptachlor on the Flavor of Turnips, Parsnips, and Carrots[1]

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ABSTRACT

Turnips, parsnips, and carrots grown in untreated soil or soil treated with 3 or 6 pounds per acre of heptachlor, or 5 or 10 pounds per acre of chlordane were evaluated for flavor. In one year an additional dosage of 20 pounds per acre of chlordane was applied to soil in which carrots were grown. Results of the palatability evaluations indicated that soil treatment with heptachlor or chlordane in the dosages reported had no adverse effect on the flavor of these three vegetables.

The application of chlordane or heptachlor has been employed in the growth of many food crops. It has been reported that applications of chlordane or heptachlor in the production of certain fruits and vegetables did not adversely affect the flavor of peaches, raspberries, strawberries, beets, cabbage, carrots, cucumbers, radishes, rutabagas, squash, and turnips (Birdsall *et al.* 1957, Boswell 1955, Foster *et al.* 1956, Hard & Ross 1959, Stitt 1953). However, reports of other studies on the effects of chlordane (Birdsall *et al.* 1957, Boswell 1955) and heptachlor (Birdsall *et al.* 1957) indicate that off-flavors were found in carrots, lima beans, potatoes, pumpkin, sauerkraut, and tomatoes.

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This paper reports results of a study of palatability conducted in cooperation with the Entomology Research Division of the U. S. Department of Agriculture to establish more definitely whether the flavor of such underground crops as turnips, parsnips, and carrots was affected by soil treatment with heptachlor or chlordane.

PROCEDURES.—In 1956 turnips of the Purple Top White Globe variety and parsnips of the Hollow Crown variety were grown by the Entomology Research Division in plots located at Beltsville, Maryland. The two vegetables were grown in the same plots which were arranged in randomized blocks to provide four replications of the untreated and of each treated sample of both turnips and parsnips.

Dosages of 5 or 10 pounds per acre of chlordane or 3 or 6 pounds per acre of heptachlor were mixed with 5-10-5 fertilizer, applied to the soil at the rate of 1,000 pounds per acre, and disced in before planting time. The untreated control plots were fertilized only. Both crops were planted May 22, 1956; turnips were harvested November 8; parsnips, November 26.

In 1958 carrots evaluated were Germain's #48 Improved Long Type variety grown at Norwalk, California, by the Entomology Research Division Agricultural Experiment Station. Field plots were replicated twice for the untreated sample; however, only one plot was prepared for each of the treated samples. Chlordane and heptachlor treatments as used for turnips and parsnips were repeated with carrots. In addition, 20 pounds per acre of chlordane was included among the treatments to determine whether off-flavors would develop since it is possible that accidental over-dosage or accumulated dosages may reach this amount.

Carrots were planted April 10 after appropriate treatments were applied to the soil April 9. Plots were irrigated six times during the growing period with the irrigation water flowing from the untreated to the treated plots to avoid contamination of the control samples. Commercial 16-8-4 fertilizer was applied at the rate of 1,000 pounds per acre on May 16. Carrots were harvested July 7, 1958.

In 1959 a repetition of the experiment with carrots to provide replicated field plots was carried out in South Carolina. Carrots of the Chantenay variety were grown by the Entomology Research Division at their Agricultural Experiment Station in Charleston, South Carolina. Field plots were arranged in randomized blocks to provide four replications of the untreated sample and of each of the treated samples. Two dosages each of heptachlor or chlordane, the same as the previous year except for the elimination of the 20 pounds per acre chlordane dosage, were applied in the form of an emulsion in 50 gallons of water per acre. The insecticide and fertilizer were mixed into the soil to a depth of 4 to 6 inches with one trip of a double disc harrow. Carrots were seeded January 22, 1959, and harvested May 8.

As soon as possible after harvest the vegetables were delivered to the Human Nutrition Research Division laboratory. Turnips were held at 32° F. from the date of delivery for about 3 weeks during the course of the taste tests. Parsnips were stored at 32° F. for 2 months to allow for the development of sugar and characteristic sweet taste before palatability evaluations were conducted. Harvested carrots of the 1958 crop were stored 1 week and those of the 1959 crop for 3 days at 32° F. before the taste tests were started. Carrots of both the 1958 and the 1959 crop were held at 40° F. for approximately 2 weeks during the course of the palatability evaluations.

All vegetables were removed from cold storage and warmed to room temperature (70° F.) before preparation. Each sample was scrubbed, rinsed in distilled water, and dried. It was then pared and cut into the desired size for cooking; turnips were cut into $\frac{1}{2}$-inch cubes, parsnips sliced crosswise, and carrots cut into $\frac{3}{8}$-inch dice. Carrots were also served raw as strips. The cleaned carrots were cut into top and bottom halves and put into two lots. These halves were cut lengthwise into $\frac{3}{8}$-inch strips and chilled 10 minutes in a refrigerator until tested by the panel.

Each sample of a specific vegetable was cooked in a covered enamel pan in a minimum amount of boiling distilled water for a predetermined period; 18 minutes for turnips, 15 minutes for parsnips, 18 minutes for 1958 carrots, and 10 minutes for 1959 carrots. Carrots cooked in 1959 were less mature and thus more tender than those cooked in 1958. Drained cooked turnips were then mashed with a potato masher and stirred to make a homogeneous sample. Parsnips and carrots were drained and served to the panel members without further preparation. For each sample of raw carrots two strips from the top halves and two from the bottom halves were randomly selected to insure the tasting of all portions of the carrots by each panel member.

Palatability evaluations were made by five members of the Human Nutrition Research Division staff in a room separate from that in which the samples were prepared. The panel members were not connected with the preparation in any way. Individual portions of each raw and cooked vegetable sample were served in coded white dishes to the panel members at 5-minute intervals. Evaluations were made at individual tables so arranged that panel members could not observe each other's reactions.

Flavor was evaluated on 5-point scales on which 5 represented natural, no off-flavor; 4, slight off-flavor; 3, moderate off-flavor; 2, strong off-flavor; 1, very strong off-flavor. Similar scales were used in evaluating odor of turnips and parsnips.

The experimental design used in the palatability evaluations of turnips, parsnips, and 1959 carrots was a Youden square (Cochran & Cox 1957) which consisted of five sessions at each of which four different samples were served. The design provided four replications for each of the five treatments, each replication corresponding to a field block. Another Youden square design was used for the serving order of 1958 carrots in which two replicated lots of the control sample provided for the seven treatments required for this design. Samples of four treatments were served at each of seven sessions. The design yielded eight scores for the untreated sample and four for each treated sample. Raw and cooked carrots were evaluated in separate experiments.

RESULTS AND DISCUSSION.—Flavor and odor scores for cooked turnips, cooked parsnips, and raw and cooked carrots are presented in table 1.

Each mean score for flavor of turnips or parsnips

Table 1.—**Mean odor and flavor scores[a] for turnips, parsnips, and carrots grown in untreated plots and in plots treated with heptachlor or chlordane.**

Treatment	Dosage (Lb./A.)	Turnips		Parsnips		Carrots			
						Flavor—Raw		Flavor—Cooked	
		Odor[b]	Flavor[c]	Odor	Flavor	Calif.	S. Car.	Calif.	S. Car.
Untreated	0	4.9	4.2	4.8	4.4	3.5	4.0	3.2	4.2
Heptachlor	3	4.8	4.6	4.8	4.3	3.8	3.6	4.0	4.4
	6	4.8	4.4	4.8	4.4	4.0	4.2	4.1	4.4
Chlordane	5	4.8	4.5	4.8	4.0	4.5	3.7	3.8	4.6
	10	4.6	4.5	4.8	4.3	3.7	3.9	3.8	4.1
	20	—	—	—	—	4.0	—	3.9	—
Test difference[d]		0.4	0.5	0.6	0.7	1.2	1.0	0.6	1.0

[a] Means for untreated carrots, 1958, were based on 40 scores (8 scores for each of 5 judges); other means were based on 20 scores (4 scores for each of 5 judges).
[b] Score of 5 represents natural, no off-odor; 1, very strong off-odor.
[c] Score of 5 represents natural, no off-flavor; 1, very strong off-flavor.
[d] The difference between two means is significant at the 5% level when it equals or exceeds the test difference (Duncan & Bonner test 1954).

grown in soil treated with heptachlor or chlordane was not significantly different from the mean flavor score for the untreated sample indicating that off-flavors did not develop. Mean scores for odor of turnips and parsnips were higher in all instances than those for flavor and no off-odors were found. All scores for these two vegetables were 4.0 or above indicating the presence of only slight off-odor or off-flavor in some samples.

In both years, 1958 and 1959, the mean score for flavor of raw carrots grown in untreated plots was not significantly different from any of the mean scores for flavor of samples grown in soil treated with heptachlor or chlordane.

In the 1958 evaluation of cooked carrots each of the mean flavor scores for samples from treated plots was significantly higher than the mean score for flavor of carrots from untreated plots. In 1959, however, cooked carrots prepared from samples grown in treated plots received a mean score for flavor which was not significantly different from any of the mean flavor scores received by the carrots grown in untreated plots.

No effect on flavor of carrots either raw or cooked was indicated by soil treatment of chlordane at 20 pounds per acre.

The flavor of turnips, parsnips, and carrots was not adversely affected when any of these vegetables were grown in soil treated with 2 levels of heptachlor or 2 or 3 levels of chlordane.

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Susceptibility of Certain Stages of the Rice Weevil[1] to a Methallyl Chloride Fumigant Formulation in Wheat of Various Moistures[2]

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ABSTRACT

Adult rice weevils (*Sitophilus oryza* (L.)) were fumigated with an 80–20 (by volume) formulation of carbon tetrachloride-methallyl chloride in wheat at 11.0%, 12.5%, and 14.0% moisture for 48 hours at 80° F. Eggs, first and third instar larvae, and pupae were fumigated in 12.5% and 14.0% wheat.

Most of the insects killed by fumigation had died within 5 days. There was no apparent knock-down with subsequent recovery.

LC_{50} values with upper and lower fiducial limits and b (slope) values with upper and lower fiducial limits were calculated for 10-day mortalities. LC_{90} values were estimated from plotted curves.

LC_{50} order of susceptibility from least to greatest at 12.5% moisture was: third instar larvae<first instar larvae=eggs <pupae=adults; at 14.0% moisture: third instar larvae<first instar larvae<pupae<eggs<adults. LC_{90} order of susceptibility from least to greatest at 12.5% moisture was: third instar larvae<first instar larvae=pupae=eggs<adults; and 14.0% moisture: third instar larvae<pupae<first instar larvae<eggs <adults.

Carbon tetrachloride-methallyl chloride (80-20) was slightly more effective against adults than carbon tetrachloride-carbon disulfide (80-20) at LC_{90}, but about equal at LC_{50}.

One of the essentials for better stored-grain fumigation is to find a fumigant effective against insects that infest kernels internally as well as externally. Literature on fumigant effects on internal forms is limited. Briejer (1938), Lindgren *et al.* (1955a, 1955b) and others have reported the effects of methallyl chloride on the egg, larval, and pupal stages of certain species. Adkisson (1957) reported the susceptibilities of the life stages of rice weevil, including each larval instar, to two fumigants.

This investigation was to determine the susceptibilities of certain life stages of the rice weevil to an 80-20 formulation of carbon tetrachloride-methallyl chloride (CCl_4-MC) in wheat of various moistures.

MATERIALS AND METHODS.—Hard red winter wheat, 1952 crop, variety unknown, was obtained from the Commodity Credit Corporation for fumigation tests. Prior to use the wheat was cleaned to less than 1% dockage and tempered to moistures of 11.0%, 12.5%, and 14.0%.

Adult and immature rice weevils were reared in 13.0% moisture wheat in a rearing room at 80° ± 2° F. and 70% ±5% relative humidity. One hundred parent weevils from laboratory cultures were placed in each of several 1-quart wide-mouth Mason jars containing 100 grams of wheat at 13.0% moisture. Jars were covered with screened lids lined with filter paper. After a 3-day oviposition period, parent weevils were removed. Eggs laid during this period were allowed to develop at 80° F. and 70% relative humidity. Cultures were started at 3-day intervals to provide a constant supply at various ages.

Adult insects began emerging approximately 33 days after start of the oviposition period. For tests, adults were obtained from cultures approximately 49 days after start of the oviposition period. Eggs, first and third instar larvae, and pupae were the immature forms studied. Each of these forms was obtained using a schedule formulated by Adkisson (1956). By dissecting infested kernels and measuring larval head capsule widths, he determined the time interval from oviposition when the greatest percentage of a given form was present within a sample of wheat. Samples from cultures of eggs, first instar larvae, third instar larvae, and pupae were fumigated 3 to 4, 8 to 9, 15 to 16, and 25 to 26 days after start of the oviposition period, respectively. O'Donnell (1956) reported similar data for larval instars when rice weevils were reared in wheat at 80° ± 2° F. and 70% ± 5% relative humidity.

An 80-20 (by volume) formulation of carbon tetrachloride-methallyl chloride was prepared by adjusting the temperature of each compound to 77° F. and measuring the required amounts with a 100-ml. graduate cylinder. A total of 500 ml. was formulated and stored at 40° F. in an amber glass bottle with a noncorrosive screw-type cap.

One-gallon, narrow-mouth glass jars with self-sealing Mason lids were used as fumigation chambers. Fumigant was applied to a folded half-sheet of 9 cm. filter paper suspended on an L-shaped, fine-gauge steel wire soldered to the inner surface of the lid.

Fifty adult insects, approximately 14 to 21 days old, were placed in 60-mesh, bronze, wire-screen cages (3 inches long and $\frac{7}{8}$ inch in diameter). Each cage contained 10 grams of wheat with the same moisture as that used in the fumigation chamber. Wax-coated corks were used to stopper the cages. Wheat, containing the desired immature form in sufficient quantity to provide two 10-gram samples per fumigation chamber, was thoroughly mixed by making three passes through a Boerner grain divider. Ten-gram samples, taken at random, were placed in wire cages and stoppered with wax-coated corks.

Two cages of test insects were placed in each chamber midway in a mass of wheat with long axes of the cages horizontal. Each chamber contained a total of 2,000 grams of wheat. One hundred adult weevils were used per chamber. Numbers of immatures per chamber varied

[1] *Sitophilus oryza* (L.).

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among tests. Chambers containing wheat and test insects were stored for approximately 24 hours at 80°±2° F. prior to fumigation.

A Micro Syringe (Pedersen 1959) with 0.25-ml. capacity and capable of delivering accurately, increments of 0.0001 ml. was used to introduce fumigant. Fumigant at 77° F. was placed in test chambers by partially lifting the lid and inserting the syringe needle until it touched the filter paper fold suspended approximately 2 inches within the chamber. Fumigant was placed on the filter paper by plunger action of the syringe. The syringe was withdrawn and the chamber immediately sealed.

Test insects in chambers were exposed for 48 hours at 80°±2° F. after which they were removed from the chambers and aerated for 1 hour.

After aeration, percentage mortality of adults was determined and recorded as "0-day mortality." Criterion for death was total immobility. Any insect showing any movement whatsoever was considered alive. Insects from each cage were placed in 1-ounce tin salve boxes containing 10 grams of clean, uninfested wheat at 13.0% moisture. Each salve box was covered by a screened lid held in place by an identification tape.

All salve boxes for a given fumigation test were buried in 15 pounds of wheat, 13.0% to 14.0% moisture, in a 30-pound capacity can. The lid was sealed with masking tape to exclude other insects and to maintain moisture. After 5 days' storage at 80°±2° F., the mortality was again determined. The insects were then restored, as previously described, and final mortality determined 10 days after exposure period.

Ten-gram samples of fumigated wheat containing immature forms were aerated 1 hour and then placed in 1-ounce tin salve boxes with screened lids. Samples were buried and stored in wheat in the same manner as for adults. Samples were removed and number of emerged adults recorded at approximately 32 days from the start of the oviposition period for a given immature form. Five counts were made thereafter at intervals of 5 days. Mortality of immatures was determined by subtracting the total number of adults emerging from each treated sample from the number emerging from its respective control sample.

Three separate fumigation tests were made with each insect form in 12.5% and 14.0% moisture wheat but only adult weevils in 11.0%. Mortalities for treated samples were not corrected for natural mortality inasmuch as average total percentage mortality for controls did not exceed 2.0% in any test. A computer program, described by Sokal (1958), to process dosage-mortality data by the method of maximum likelihood with an IBM 650 Magnetic Drum Data Processing Machine, was used to statistically analyze the dosage-mortality data.

RESULTS AND DISCUSSION.—Per cent mortality of adults after 0, 5, and 10 days is reported in table 1. There was, in general, only a slight difference in the average total percentage mortality for the 5- and 10-day counts. Between the 0- and 5-day mortalities, however, there was an appreciable difference, especially near the center of the effective range of the fumigant, but this difference decreased with increased moisture. Data indicate that by 5 days after fumigation, under the conditions of this study, most of the insects that will die as a result of the fumigation have been killed. These results show the same rapid, lethal effect as was found by Breijer (1938) with granary weevils, *Sitophilus granarius* (L.), and Richardson (1945) with cadelles, *Tenebroides mauritanicus* (L.), using methallyl chloride. Some fumigants, for example CCl_4-CS_2 (80-20), require an appreciably longer period before effective mortality (delayed effect) can be determined (Whitney & Harein 1959). There was no evidence of an initial knock-down with subsequent recovery. Instead, the data suggest that some insects that will eventually die are still active on the 0-day mortality count. In contrast, some fumigants may cause a knockdown effect with a subsequent recovery (Whitney & Harein 1959).

Table 1.—Per cent mortality[a] of adult rice weevils when exposed to CCl_4-MC (80-20) for 48 hours in wheat at three moisture levels.

Dosage (μl./2000 gm.)	Days Following Exposure								
	0	5	10	0	5	10	0	5	10
	(Moisture Content of Wheat)								
	11.0%			12.5%			14.0%		
30	9.8	20.6	20.7						
35	9.4	19.1	19.1						
40	14.7	38.8	42.1						
45	24.5	50.0	52.0						
50	39.4	71.6	76.9	6.4	6.4	6.4			
55	61.3	79.7	82.0	10.1	11.8	13.4	8.4	9.0	10.4
60	74.3	90.9	91.2	18.9	28.4	29.4	16.6	17.4	18.6
65				32.2	54.1	56.2	16.6	20.9	20.6
70				54.5	69.4	20.0	19.4	25.2	25.8
75				68.0	83.7	86.3	31.3	42.2	45.6
80				88.4	95.3	96.0	48.0	55.7	57.2
85							63.6	67.4	67.1
90							69.0	76.4	78.9
95							85.0	93.8	94.6

[a] Average of three tests.

Table 2 is a compilation of LC_{50} values, b values (slopes), the upper and lower fiducial limits of LC_{50} and b at 0.05 probability, and estimated LC_{90} values for adult and immature forms of rice weevil. LC_{90} values were estimated from plotted curves. Data are for 10-day mortalities.

When the LC_{50} values and their upper and lower fiducial limits of the various stages are compared, the order of susceptibility from least to greatest was: 12.5% moisture—third instar larvae < first instar larvae = eggs < pupae = adults; 14.0% moisture—third instar larvae < first instar larvae < pupae < eggs < adults.

A direct relationship existed between moisture content of wheat and amount of fumigant required for a given per cent mortality. Each form fumigated in wheat at 14.0% required more fumigant than at 12.5% to attain an equal mortality.

At both moistures, third instar larvae were least susceptible. Innumerable factors might be responsible for one stage of an insect being more or less susceptible than another. Sun (1947) and others believe the susceptibility of insects depends mainly on the rate of respiration. Lindgren (1935), however, found that this relationship did not apply in all cases. Sun further stated that "physical factors" not only affect respiration but also absorption, adsorption, diffusion, permeation, volatility, chemical reaction, water loss, et cetera, and that these factors more or less affect the toxicity of fumigants to insects.

Eggs and first instar larvae are classed as equal in susceptibility when fumigated in 12.5% moisture wheat be-

Table 2.—Dosages of an 80–20 (by volume) formulation of carbon tetrachloride-methallyl chloride required to kill 50% and 90% of rice weevil adult and immature forms when exposed for 48 hours at 80±2° F. in 2,000 grams of wheat at three moisture levels.[a]

Moisture of Wheat (%)	Stage	Slopes			μl./2,000 Gm. of Wheat			
		b_l	b	b_u	LC_{50_l}	LC_{50}	LC_{50_u}	LC_{90} (est.)
11.0	Adult	5.42	7.76	10.10	39.03	41.94	44.73	61.4
12.5	Egg	8.29	10.89	13.49	75.97	79.62	83.13	104.0
	1st instar	5.65	9.35	13.05	74.39	81.06	87.52	111.0
	3rd instar	11.39	15.51	19.63	100.47	104.35	108.27	126.0
	Pupa	4.60	5.11	5.61	58.67	61.21	63.51	109.0
	Adult	14.82	15.96	17.10	63.43	64.03	64.63	77.0
14.0	Egg	8.28	9.24	10.20	91.55	93.43	95.27	129.0
	1st instar	10.75	12.69	14.62	111.34	114.05	116.68	144.0
	3rd instar	7.37	11.12	14.90	130.73	137.12	145.22	180.0
	Pupa	6.60	7.24	7.87	98.76	100.94	102.98	153.0
	Adult	8.40	10.76	13.13	72.86	75.58	78.43	101.0

[a] Mortality counts 10 days after fumigation.

cause the LC_{50} upper and lower limits of the egg and first instar larvae, respectively, overlap. Pupae and adults were equally susceptible and less resistant than eggs and first instar larvae. Order of susceptibility varies with respect to moisture for eggs and pupae. There is also an apparent separation in the susceptibilities of the various forms in the 14.0% moisture wheat to form five separate groups, *i.e.*, there was no overlap of susceptibility.

Although the upper and lower limits of LC_{90} were not calculated, the orders of susceptibility from least to greatest appear to have been: 12.5% moisture—third instar larvae < first instar larvae = pupae = eggs < adults; and 14.0% moisture—third instar larvae < pupae < first instar larvae < eggs < adults. The apparent separation in susceptibility is again present at 14.0% moisture. Orders of susceptibility changed from those at LC_{50}. Pupae became comparatively more "resistant" at LC_{90} as evidenced by the decidedly smaller b (slope) at both moisture levels. Slopes for other stages do not exhibit any definite pattern.

Upper and lower fiducial limits of b and LC_{50} show that third instar larvae had the greatest overall variability; second were first instar larvae followed by eggs, adults, and pupae, respectively.

By comparing CCl_4-MC (80-20) data from this study with those for an 80-20 formulation of CCl_4-CS_2 (O'Donnell 1958), an estimation of relative effectiveness can be obtained (table 3). Based on O'Donnell's studies in this laboratory under essentially the same conditions, CCl_4-MC (80-20) was found to be slightly more toxic to adult weevils than CCl_4-CS_2. LC values for 11.0% moisture wheat show no differences between the two formulations. In 12.5% wheat, CCl_4-MC (80-20) required 6.7 μl. less fumigant at LC_{50} and 27.3 μl. less at LC_{90}. Both formulations required about the same dosage for LC_{50} in 14.0% wheat but for LC_{90}, CCl_4-MC (80-20) required 32.2 μl. less.

Carbon tetrachloride-methallyl chloride also appears relatively toxic to immature forms; however, comparable data with CCl_4-CS_2 (80-20) are not available. At LC_{90} the least susceptible immature form, third instar larvae, required 1.64 and 1.78 times as much fumigant as was required to kill 90% of the adults at 12.5% and 14.0% moisture, respectively.

Table 3.—Comparison of LC_{50} and LC_{90} values for 80-20 formulations of carbon tetrachloride-methallyl chloride and carbon tetrachloride-carbon disulfide when used as a fumigant for adult rice weevil in 11.0%, 12.5%, and 14.0% moisture wheat.[a]

80–20 Fumigant Formulation	LC	μl./2,000 Grams of Wheat		
		11.0%	12.5%	14.0%
Carbon tetrachloride-methallyl chloride	LC_{50}	41.9	64.0	75.6
	LC_{90}	61.4	77.0	101.0
Carbon tetrachloride-carbon disulfide[b]	LC_{50}	40.1	70.7	75.0
	LC_{90}	60.4	104.3	133.2

[a] 48-hour exposure at 80±2° F.
[b] O'Donnell (1958).

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Cotton Insects in Nicaragua[1]

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ABSTRACT

The cotton-insect problem in Nicaragua is in many respects similar to that in the United States. The most serious pests are the boll weevil (*Anthonomus grandis* Boh.) and *Sacadodes pyralis* Dyar, a Phalaenid which bores into bolls. The boll weevil passes the dry season between crops in old cotton fields. Reproduction apparently ceases during this time. Early infestations in young cotton were heaviest in fields planted before the usual date. Peak infestations occurred when the crop was approaching maturity. The life cycle is similar to that in the United States. *Sacadodes* also was generally a late-season pest, and was particularly serious in late-planted fields. Infestations of both insects were reduced by means of insecticides, but increases in yield were small.

Prior to 1950–51 cotton production in Nicaragua was insignificant, amounting to 5,000 bales or less annually. When the war in Korea began, United States stocks were dangerously low and competition arose from other sources which could supply cotton similar to U. S. upland. Prices for non-U. S. grown cotton rose to approximately 75 cents per pound. As a result, Nicaragua and other countries with suitable soil and climate engaged in a mad rush to increase acreage in such a profitable crop. In 1954–55 Nicaraguan production reached 205,000 bales. By this time the shortage of United States cotton had changed to a surplus and prices for Nicaraguan cotton had declined to approximately 36 cents. Acreage leveled off and has remained fairly constant since. The price in 1958–59 was down to about 24 cents.

When acreage of cotton began increasing, demand arose for research on insect enemies of the crop. The information subsequently obtained has utility for entomologists in the United States as well as in Nicaragua, since many of the insect problems are similar. Four of the most important U. S. cotton pests are also problems in Nicaragua. Information from each country may thus supplement that from the other in many respects. Since the growing season in Nicaragua is later than in the United States, Nicaragua could even be used as an off-season base for research.

The information presented here was obtained during 2 years with the U. S. Department of State, International Cooperation Administration, (Point 4) as entomological adviser to the Minister of Agriculture and Animal Industry in Nicaragua. The author's duties included any entomological problem of plants, animals, or man. Thus, an opportunity was offered to obtain considerable information on cotton pests.

COTTON CULTURE.—Cotton production in Nicaragua is restricted mainly to the western coastal plains, where the volcanic soil is deep, friable, and extremely fertile. High yields of American varieties are produced even without fertilization. Fields tend to be large, and mechanization is progressing rapidly.

Western Nicaragua has roughly a 6-month dry season, from about November 15 to May 15. The remaining 6 months are very wet. Temperatures are moderately high the entire year, averaging slightly lower during the wet season. Nearly steady strong winds blow during the dry season.

Cotton is planted about the middle of the wet season, in late July, and matures during the dry season, in late December. Fruiting is often profuse even after rains stop in November, because the soil is very retentive of moisture. Cotton makes extremely heavy vegetative growth in most fields. The plants commonly fill the middle of the rows, and frequently reach 6 feet or more in height. Fruiting is heavy but many squares and young bolls are usually shed.

THE INSECT PROBLEM.—Previous publications on the cotton-insect problem in Nicaragua include those by Swain (1952) and by Miner (1956). Insects constitute a major factor in production, but the problem is not uniform geographically or seasonally. Seasonal peak populations of the serious pests came late in the growing season for cotton, unless the crop was planted early. Thus, the early crop of fruit was generally safe, before insects became numerous, but the late crop was often subject to extremely heavy attack.

Insecticidal controls as carried out by the average grower have not yet advanced to the point of timing according to infestation. In fact, there was a distinct tendency to stop applications in late October, when serious insect infestations were just starting. The most common system of timing was to make weekly applications starting with setting of the first squares, or earlier. This practice was continued throughout the season, insofar as weather and other factors allowed. Often it was a matter of exhausting the supply of dust or money part way through the season, at which time applications ceased.

The use of ground application machinery was made difficult by mud and heavy vegetative growth. Maintenance of insecticidal deposits on the plants was also difficult owing to frequent rains. Airplanes were widely used as a partial solution to the problem. Pilots and

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planes were nearly all from the United States.

THE BOLL WEEVIL.—The most injurious insect pest is the boll weevil (*Anthonomus grandis* Boh.). It occurs throughout the cotton-producing area and can build up to tremendous numbers, especially early in the dry season as the crop approached maturity.

Brief life-history studies were conducted in the field during June and July, 1956. Squares that had fallen during the previous 24 hours were collected and placed on the soil under plants, and the date of emergence was determined for 101 adults. The average period in the square after it dropped was 11.5 days, ranging from 9 to 15. During the same period, using different squares, records were taken on the number of days between puncture and dropping of the square. The average period was 6.3 days for 39 squares. If this is added to the above figure of 11.5 days for the sake of an estimate, a life cycle of about 18 days is indicated. The preoviposition period was not noted.

In another experiment the date of emergence from the square was noted for 13 adults, where the actual date of deposition of the egg had been recorded. The period in the square ranged from 14 to 22 days, and averaged 17.7 days. Thus, the life cycle appeared to correspond closely with that in the United States during favorable summer weather. Temperature records for the above experiments are not now available, but at this season the daily minimum ordinarily would be about 70° F., and the maximum about 90° F.

Seasonal infestation records were obtained in 1955 on 28 fields in the vicinity of Managua and of Leon. These data, along with numerous other records not quoted, indicate that early infestations are rare in cotton planted near the normal date. One of the fields, planted quite early, was squaring freely on August 15, the date of first inspection. Fifteen per cent of the squares were punctured, and the infestation rose rapidly thereafter. After 3 weeks, infestation records were discontinued, since the field was evidently a hopeless case as far as control was concerned.

Of the other 27 fields, none were squaring on August 15. Three of these fields reached approximately 10% infestation near October 1. Ten more reached 10% infestation on November 1 or a little earlier. The remaining 14 fields reached 10% infestation on various dates in late November or early December. By mid-December many fields had counts in the 90's, with others soon to follow. Records for 1954 near Managua and Masaya, and for 1956 near Managua were very similar to these records. Data taken in previous years by Swain (1952) are also in close agreement.

After infestations reach their peak in December, the cotton matures and populations become static. If the plants are not destroyed, however, the boll weevils remain in the field. With the onset of the extremely hot, dry, windy weather of January or early February, numbers suddenly drop to a relatively low level. During the remainder of the dry season the decrease in numbers continues, but at a much lower rate.

This period spent in old fields between cropping seasons corresponds to the hibernation period in the United States. Plants generally do not die during the dry season, although little fruiting occurred and vegetative growth was woody. Even when plants were chopped off at the soil surface, some new growth was produced. A few weevils could be found on these plants all through the dry season. Extensive search was made for other sites of survival, but none were found except wild cotton, which was of rare occurrence and can be largely ignored. Repeated experiments designed to carry adults through the dry season in dead plant residues, such as cornstalks, met with failure.

Records on survival in an old cotton field were made during the latter part of the dry season of 1954–55, starting March 1. The initial population crash had already occurred. Once each week, one man searched the field for 1 hour and counted all the weevils he could find. From a high of 157 weevils on March 9, the count dropped with considerable regularity to a low of 15 on April 27. Rains began shortly thereafter and counts consistently increased. It is not known whether the increase was caused by reproduction or by greater activity.

Reproduction apparently ceased during the dry season. In order to check this point, a plot of cotton was grown during the dry season in 1955 by means of irrigation. Fruiting was constant, although limited. Numerous weevils were present in the plot throughout the experiment. There were far more than enough to puncture all the few squares present, but the per cent of punctures remained low. The highest count was on March 7, when 35% punctured squares were recorded. On the same date, in a nearby unirrigated plot left from the previous year, less than 1% punctures were found in the few squares present. Frequent punctures of terminal growth were noted. No larvae or pupae were ever found in fallen squares in either plot. This cessation of reproduction may be caused by the sexual diapause reported by Brazzel & Newsom (1959).

When the new crop of cotton emerges, any weevils that have survived the dry season promptly move in. The details of this movement were not discovered, but the fact of migration at planting time and for some time thereafter was demonstrated experimentally. In July 1956 careful daily hand collections were made of all weevils to be found in small plots of young cotton. No regular decrease in number of weevils occurred from day to day, indicating a constant replenishing of numbers from outside the plots. Tanglefoot screens located about 100 feet from the plots in the direction of the nearest old cotton field failed to catch any flying weevils, however. Bottoms of the screens were about 3 feet off the ground.

In every recorded case, heavy early-season infestations were in cotton planted well before the usual date, presumably because the bulk of surviving weevils concentrated on the first cotton to come up. Nearness to old fields probably was also a factor.

THE FALSE PINK BOLLWORM.—*Sacadodes pyralis* Dyar (Phalaenidae), locally known as the false pink bollworm, is second in importance. The larva is about the size of a small bollworm and is marked with a series of large red spots on the dorsum. Its damage is restricted to certain areas and it is not serious every year, but the intensity of local damage often exceeds that of boll weevil. It is more difficult to control than is the boll weevil.

This insect was first reported in Nicaraguan cotton by Dr. Ralph B. Swain in 1951. Since it was initially found dispersed over a wide area, it had probably been in the

country for some time. The most easily available account in English is by Withycombe (1926). An extended account of its life cycle and control was given by Hernandez (1956). Whitcomb (1954) reported on some of its biology and also included several excellent photographs of larvae.

The damage is caused by larvae boring into bolls, generally as first instars. Ordinarily the larva completes its entire development within one boll, but occasional larvae move to additional bolls. Squares may also be damaged, but bolls are preferred and are generally available during the normal infestation period. Observed food plants included only wild or cultivated cotton.

The life cycle of this insect is normally about 6 weeks, but it may be greatly prolonged by pupal diapause. The adult hides on the underside of foliage by day and flies by night. It is strongly attracted to light, especially ultraviolet. Moths were often observed clinging to baggage of international travelers at the Managua airport, having been attracted by the lights. The moths take no food, so far as could be determined, although literature references differ on this point.

The eggs, numbering about 200, were deposited singly on bracts, branches, or in the axils of branches. The incubation period was 5 to 6 days. Eggs deposited during the dry season did not ordinarily hatch, but could be made to do so by artificially increasing the humidity.

Behavior of the first instar larva when entering a fruit was studied in detail, since insecticides were most effective at that time. After hatching, the larva was observed to wander about for periods approaching an hour before starting to enter. It then spent additional time spinning silken threads between the fruit and the peduncle. In the case of squares this connection caused the damaged fruit to hang to the plant, which doubtless was an aid in later movement to another fruit. Bolls generally did not fall, in any case.

After starting an entrance the first dozen or so bites were not swallowed but expelled. If the hole was on the upper side, as was usual, the castings were deposited in a circle around the hole. Once the head was well inside, the bites were swallowed. The actual operation of entering required from 1 to several hours up to the time the larva was out of sight. Entrances generally were in the basal third of the fruit. The larva later maintained this opening to the outside, enlarging it as growth occurred.

After 15 to 18 days the larva was full grown and descended to the soil. Here it burrowed down for 1 or 2 inches and formed a pupal cell. A hard shell of soil formed about the cell. The prepupal stage lasted about 4 days and the pupal stage from 2 to 3 weeks. The adult then emerged from the soil, and passed a brief preoviposition period of 1 to 2 days.

Larvae that matured during the dry season had a prolonged pupal period in the soil. Since the seasonal history of the following year started with emergence of these aestivating pupae, extensive but unsuccessful efforts were made to determine the factor responsible for the break in diapause. Late in the dry season, 82 aestivating pupae were buried at various depths in the soil, and the soil was then irrigated frequently. None of these pupae ever emerged, even after rains began, but the majority were still alive after 6 months. To all appearances they were dead, but under magnification the dorsal aorta could be seen pulsating slowly. The slowest rate observed was an average of once in 13 seconds. Other pupae were kept air dry on a desk. One emerged, deformed, after 3 months. Others were kept dry in a screened insectary. After slightly more than a year, one female emerged, apparently in good condition.

Field notes and light-trap data suggested that in nature the break in diapause was straggling, and not a sharp response to any one condition. The initial emergence of moths in considerable numbers was about 2 months after rains began.

Seasonal history was followed by means of light traps and field infestation records. Light-trap data showed that moth flights continued into the middle of the dry season, but very few moths reproduced at this time. Only occasional moths were found during the second half of the dry season. Resumption of heavy flights came well into the next wet season, in early July. This was shortly before cotton-planting time. Flights continued to late August, or about the time when cotton started fruiting, after which numbers dropped. About mid-October or early November, the number of adults flying increased again, and a peak was reached in early January. In 1955 this peak came on January 11, and in 1956 on January 2. Sometime in early February the flights dropped abruptly, and only a few moths could be taken during the remainder of the dry season.

Larval infestations followed closely on the above schedule of moth activity unless prevented by hot, dry weather or by absence of cotton. The first flights in July and August found little cotton fruiting. Heavy infestation of extra early cotton was noted several times during this period. Probably the lack of infestation during the early part of the growing season (September and October) was due to the fact that this first brood of moths emerged a few weeks too early. Larval infestation began again in October or November, and infestations then rose steadily and often rapidly. Late-planted cotton, maturing during the worst of this danger period, often had 100% of the late bolls destroyed.

There were indications that certain areas served as focal points for the population. While infestations were widespread, by far the greatest problem was in the lowlands near Lake Managua and the Tipitapa River, which emerges from this lake. The region contains much low swampy soil that has a high moisture content during a large part of the dry season.

One field in the area, which was kept under intermittent observation, had squares still forming during the middle of the dry season. There were also recent entrances by *Sacadodes*, long after infestations were absent elsewhere. High soil moisture might shorten the pupal diapause, and might increase the atmospheric humidity sufficiently to permit hatching of eggs during the dry season.

MISCELLANEOUS INSECTS.—Other insects are distinctly of minor importance. *Alabama argillacea* (Hbn)., the cotton leafworm, was a common pest but generally did little damage because insecticides were so commonly used for other insects. If a field was not being treated, severe infestations could develop with extreme rapidity. Generally the cotton leafworm was present in injurious

Table 1.—Square production and insect damage as influenced by insecticide treatment. Managua, Nicaragua. 1955.

No.	Treatment[a]	Rate Actual per Acre (Lb.)	Total Squares (2 Rows)	Boll Weevil Punctured (%)	*Sacadodes* Entrances (%)	Seed Cotton per Acre (Lb.)
1	3–5 dust, (BHC-DDT)	0.53 BHC	69	94	7	1,026
2	3–10 dust, (BHC-DDT)	.53 BHC	46	89	11	1,110
3	3–10 dust, (BHC-DDT)[b]	.53 BHC	32	91	9	761
4	7–20 liquid (BHC-DDT)[c]	.44 BHC	186	88	5	1,057
5	3–5 liquid (BHC-DDT)	.44 BHC	258	79	9	999
6	3–5 liquid+2% oil	.44 BHC	106	81	2	1,123
7	Calcium arsenate dust	18.0	52	87	10	1,052
8	Endrin 2% dust	.35	219	86	5	986
9	Endrin liquid	.44	798	74	4	973
10	Dieldrin liquid	.44	109	93	5	1,008
11	Aldrin liquid	.44	180	86	7	1,026
12	Toxaphene 20% dust	3.50	77	92	8	991
13	Toxaphene liquid	3.0	85	87	6	1,114
14	Methyl parathion 2% dust[c]	.35	94	84	9	1,026
15	Methyl parathion liquid[c]	.30	242	73	9	1,128
16	Guthion liquid[c]	.30	506	51	2	1,141
17	Untreated	—	0	—	—	951

[a] Insecticides are of U. S. origin except as otherwise noted.
[b] Formulated in Nicaragua with a local clay as diluent.
[c] German origin.

numbers only after October, but in 1955 it was a problem throughout the season.

Aphids (principally *Aphis gossypii* Glov.) were frequently a minor problem late in the growing season and sometimes, as in 1954, did considerable damage. Lint was stained if the infestation came after bolls had opened. *Prodenia* species caused occasional damage to bolls. *Heliothis zea* (Boddie) was an occasional problem. Cutworms, *Feltia* and *Acroloppus* species, cut off young plants, especially in early planted fields. *Estigmene* sp., possibly the salt-marsh caterpillar (*E. acrea* (Drury)), caused occasional damage. Leaf-cutting ants (*Atta spp.*) frequently caused damage to border rows. The pink bollworm, *Pectinophora gossypiella* (Saund.), does not occur in Nicaragua.

Control Experiments.—Several field-plot experiments on insecticidal control of the boll weevil and *Sacadodes* were conducted. These experiments provided valuable information, but represent only a beginning on the problem of control. The salient features of results are discussed here more as background for possible future work than to draw final conclusions.

A field-plot experiment on insecticidal control was started in 1954, but was later abandoned because of heavy rains and wind, which caused the plants to lodge badly. Treatments could not be applied by tractor-mounted equipment then in use because of mud and fallen plants.

In 1955 another field test was carried out. The object was to test performance of numerous insecticides against the boll weevil and *Sacadodes* under a single system of timing.

Plots were treated at weekly intervals when the boll weevil infestation reached 10% or when five *Sacadodes* eggs per 100 plants were found. The plot design was a randomized block, made up of 17 treatments including a check, with four replicates. Plots were 40 ft. wide (12 rows) by 95 ft. long, making one-twentieth-manzana (0.087 acre). Fifteen-foot alleys were left between blocks and 10-foot alleys between plots. The strain Acala 29-16 was used, imported from Arizona.

Since tractor-mounted application equipment could not be used, a light motor-driven, single-spout blower-type duster, carried by two men, was utilized. The spout was aimed horizontally, about a foot below the tops of the plants. The machine was carried down each fourth row. Coverage was judged to be good throughout the foliage, despite the unorthodox method.

Sprays were applied with a modification of the same machine. A 3-gallon compressed-air sprayer with a pressure gauge was mounted on the blower in such a way that the spray was delivered into the air stream. The spout was carried about 6 inches above the tops of the plants, so that a large part of the liquid was blown above the plants and allowed to settle, as in airplane applications.

Early infestations of the boll weevil and *Sacadodes* were low. Since the aim was an insecticide test rather than an evaluation of the usual problem, weevil-infested squares were collected from another field and broadcast in quantities over the plots. Shortly after November 1 infestations of the boll weevil were sufficiently high in most plots to justify treatment, although a majority of the bolls were then safe from attack. *Sacadodes* infestations came only after the crop was nearly mature. A total of nine treatments were applied to each plot between November 7 and December 29.

The infestation count made between December 19 and 22 is presented in table 1 as an indication of relative performance of the insecticides. This count was the last made before differences between insecticides diminished owing to extremely high infestations and scarcity of squares. The table shows the total number of squares present on the two middle rows and the per cent infestation of these squares by the boll weevil and *Sacadodes*. Owing to the heavy boll weevil infestation and to limited fruiting at this time, the number of squares is considered a direct reflection of insect control. Under these extreme conditions, the per cent of punctures has meaning only in view of the number of squares present.

One notable feature of the data was the good performance of liquids as compared with dusts. Foliage in the plots was less dense than ordinarily, so that coverage by sprays was probably better than usual.

Table 2.—**Square entrances by *Sacadodes pyralis* as influenced by insecticidal sprays. Managua, Nicaragua. 1955.**

No.	Treatment	Rate Actual per Acre (Lb.)	No. Squares Entered		
			First Count	Second Count	Total
1	Guthion	0.44	22	165	187
2	Endrin	1.20	19	246	265
3	Guthion	.22	16	251	267
4	EPN	1.20	77	211	288
5	Malathion	.60	45	326	371
6	DDT	3.50	63	329	392
7	DDT	1.80	53	351	404
8	Endrin	.30	64	351	415
9	Methyl parathion	.60	63	358	421
10	Endrin	.60	108	319	427
11	DDT	.90	103	335	438
12	Toxaphene	3.50	98	386	484
13	Dieldrin	.60	87	407	494
14	Untreated	—	103	399	502
15	Methyl-demeton	.27	88	422	510
16	Dilan[a]	1.80	163	425	588

[a] 1 part 1,1-bis(*p*-chlorophenyl)-2-nitropropane and 2 parts 1,1-bis(*p*-chlorophenyl)-2-nitrobutane.

Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazine-3-(4H)-ylmethyl) phosphorodithioate) liquid and endrin liquid ranked high in insect control. Methyl parathion liquid also was moderately good except in *Sacadodes* control, where it was not expected to do well because of its short residual life. BHC-DDT mixtures were variable, with only one treatment out of six doing as well as methyl parathion. Toxaphene ranked low. In view of the wide usage of BHC and toxaphene, a clarification of their average performance is needed.

Yields were apparently influenced by the insecticides, but no attempt at analysis is made since the crop was largely set before insects became a problem. It is worthwhile to note, however, that the check, while second from the bottom, yielded a very respectable amount. The greatest increase over the check in a treated plot was 20%. In view of the extravagant use made of insecticides in Nicaragua, commonly amounting to half the total production costs, it is evident that a thorough evaluation of control procedures is needed.

Since the above field experiment did not encounter heavy *Sacadodes* infestations, a small intensive experiment on insecticidal control of first instars was conducted late in the season in plots of young cotton. Each plot consisted of one row 5 feet long. There were 15 treatments, all sprays, replicated 10 times in a randomized block. Rows were 5 feet apart, and 4 feet separated the ends of each short row. Experimentation in such small plots is considered proper with first instar *Sacadodes*, since plant-to-plant migration is negligible.

A knapsack sprayer was used, with a definite attempt to cover stems and branches. The aim was to kill first instar larvae by residues, during the pre-entrance movements. Plots were sprayed three times, on December 21, 27, and January 4. Counts of the total number of squares entered by *Sacadodes* were made in each plot on December 27 and on January 4. In table 2 the insecticides and dosages are ranked according to totals of the two counts.

The most striking feature of the experiment was the excellent showing of Guthion and endrin on the first count. Their effectiveness lessened somewhat under the heavier infestation later. EPN and malathion both ranked ahead of DDT, which was then the recommended insecticide. Since they are not used in Nicaragua, further work is suggested.

Another large field-plot test with boll weevil and *Sacadodes* was carried out in 1956. In addition to various insecticides at various rates, a trial of timing methods was included in the case of one material. Location was near that of 1955, and plot arrangement was similar. Liquids were emphasized according to their effectiveness in 1955 and to grower interest. The stand was good, foliage was dense and plants tall, which doubtless affected results as compared with the previous year. *Sacadodes* was not a problem until the crop had matured, so results apply only to the boll weevil.

Boll weevil infestations began earlier than usual, probably because a field of cotton from the previous year had purposely been left undestroyed next to the plots, and because the plots were planted a little early. Nevertheless, initial infestations were low, so again a large number of punctured squares were broadcast over the plots to assure a high infestation. Counts made between September 5 and 25 showed from 0% to 8% punctures in the various plots. On October 5 the infestation averaged 14%, and it increased regularly thereafter.

Applications were timed according to specifications shown in table 3. Treatments 1 to 15 constitute a comparison of various materials at different rates, with the same system of timing. Treatments 15 to 19 represent a test of timing methods for methyl parathion dust.

Guthion, methyl parathion, and endrin appeared to be the most effective materials, considering dosage, number of applications, infestation, and yield. Liquids in general were less effective than in 1955, possibly because of the dense foliage and resulting poorer coverage. A question was again raised concerning the effectiveness of BHC-DDT mixtures and of toxaphene. Neither was prominent in performance. An important feature of future work should be a thorough reevaluation of their field of usefulness.

In the timing trials (plots 15 to 19) there was evidence that the most profitable system was twice-weekly applications at 25% infestation. Applications made once a week at 10% infestation kept the per cent punctures at the same level with fewer applications, but probably this would not be true without artificial early infestation. Also, the yield of the 10% plots was suspiciously low. The remaining three treatments, representing more frequent and earlier applications, reduced the infestations by about 5% or 6%, which was probably not enough to pay for the extra cost.

The untreated check again yielded well, and the greatest increase over the check was less than 7%. The reasons for this situation are not presently understood. The data do not indicate a clear case of resistance, although this possibility cannot be ignored. Washing away of insecticidal deposits by frequent rains is apparently not the explanation. There are a number of other possibilities, related variously to seasonal history of the boll weevil and to tropical growing conditions.

Considering that heavy expenditures are commonly made by the growers for insecticides, that applications are

Table 3.—Boll weevil infestation and yield resulting from insecticide applications. Managua, Nicaragua. 1956.

No.	Treatment	Rate Actual per Acre (Lb.)	Timing[a]	No. of Applications	Average[b] Punctured (%)	Seed Cotton per Acre (Lb.)
1	3–10 dust (BHC-DDT)	0.53 BHC	2W10	17	29.6	1,754
2	7–20 liquid (BHC-DDT)	.40 BHC	2W10	16	37.8	1,792
3	7–20 liquid+oil	.40 BHC	2W10	16	35.6	1,533
4	7–20 liquid+sticker	.40 BHC	2W10	16	34.4	1,641
5	Toxaphene liquid	3.00	2W10	16	42.2	2,097
6	Endrin 2% dust	.35	2W10	17	27.6	2,202
7	Endrin liquid	.30	2W10	16	29.1	2,223
8		.44	2W10	16	23.5	2,118
9	Guthion liquid	.15	2W10	16	29.5	2,128
10		.30	2W10	16	21.6	2,079
11	EPN, w.p.	.44	2W10	16	32.9	2,083
12	Malathion liquid	.44	2W10	16	34.5	1,859
13	Methyl parathion liquid	.30	2W10	16	28.6	2,188
14		.44	2W10	16	26.1	2,223
15	Methyl parathion 2% dust	.35	2W10	16	22.0	2,090
16		.35	1W10	9	27.3	2,006
17		.35	2W102E	19	21.0	2,156
18		.35	2W25	12	27.8	2,206
19		.35	1WR	15	22.3	2,167
20	Untreated	—	—	—	52.8	2,083

[a] Timing systems are abbreviated as follows: 2W10 indicates two applications per week when boll weevil infestation reached 10%. 1W10 indicates one application per week when boll weevil infestation reached 10%. 2W102E indicates two applications per week at 10%, plus two early applications at 5%. 2W25 indicates two applications per week at 25% infestation. 1WR indicates one application per week regardless of infestation. (Plots were artificially infested.)

[b] Average of 10 counts. Excludes four counts made previous to first significant rise in infestations, and one after regular applications ceased and all plots were out of control.

commonly applied with disregard for the presence or absence of infestation, and that it has been difficult to obtain increased yields experimentally, it appears that an opportunity exists for considerable improvement in procedure.

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A Study of Mealybug Populations (*Dysmicoccus brevipes* (Ckl.)) in an Ant-Free Field[1]

Walter Carter

Abstract

A quantitative study of mealybug populations (*Dysmicoccus* (*Pseudococcus*) *brevipes* (Ckl.)) was made in a 24-acre pineapple field which was kept free of ants. Leaf colonies of mealybugs remained small whether the plot was sprayed or not and fluctuations were of the same order. Subterranean colonies developed in the absence of ants and the frequency and size of these colonies were roughly correlated with the incidence of scattered wilted plants. This incidence, however, was greatly reduced in strips previously occupied by field roads where plant growth was better.

The elimination of ants from pineapple fields in Hawaii has brought about profound changes in the status of the pineapple mealybug, *Dysmicoccus* (*Pseudococcus*) *brevipes* (Ckl.). The first of these changes is the reduction of populations of the typical leaf-feeding green-spotting strain to extremely low levels. The second is the maintenance of subterranean colonies that can apparently develop to large size in the absence of ant attendance, which appears essential to the well being of the typical green-spotting strain.

The biology of the subterranean colonies appears to require the occasional movement of small numbers of the insects upward to the leaves where feeding results in very

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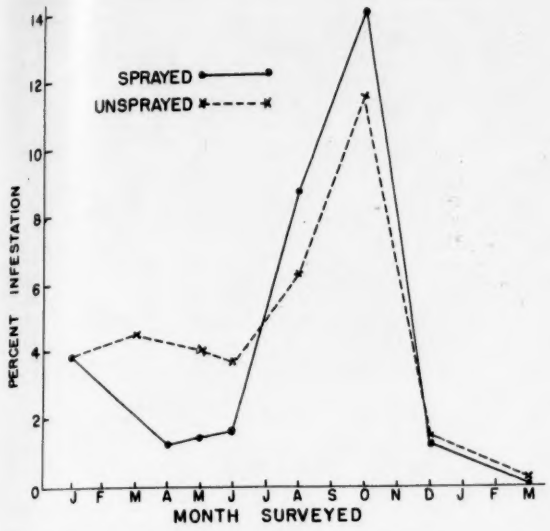

FIG. 1.—Data from sampling stations showing percentage of plants visibly infested with small colonies of from two to five mealybugs.

small atypical "pinhead" green spots, a useful diagnostic feature if the mealybugs are no longer there.

Since mealybug wilt seems to depend on the insects' feeding on the lower portions of the inner leaves, the magnitude and frequency of these upward movements are important in interpreting the role of the subterranean colonies as reservoirs from which leaf colonies may flow and cause wilt. In many fields, scattered wilted plants occur, and the etiology of this wilting is puzzling. These cases are referred to herein as "incidental wilts."

EXPERIMENTAL PROCEDURE AND RESULTS.—A field area comprising 12 plantation blocks (approximately 24 acres) was used for an experiment which sought to determine the incidence and size of mealybug colonies through out the life of the plant, the incidence of scattered wilting plants, and the relationship, if any, between both these and subterranean colonies of mealybugs.

In order to develop more than one level of the infestation for comparative purposes, the standard insecticidal spray was omitted from one-half the number of blocks in the area. Stations were established in each block for the purpose of sampling leaf populations. These consisted of 100 feet of plant line including 100 plants, and four of these were in each block. Incidental wilting was not determined by aliquot sampling but the entire field area was inspected, and wilted plants marked.

Data from the mealybug surveys are indicated in figure 1. These data are concerned only with the mealybugs visible on the above-ground portions of the plants. Percentages of plants infested only are shown, since the number of mealybugs per plant was in the range of one to three individuals.

It is clear from this record that, in the absence of ants, there were so few mealybugs infesting the leaves that spraying made no difference either to the number of plants infested or to the number of individuals per plant.

The colonies established on the subterranean portions of the plant were undoubtedly the source of these small leaf colonies. These colonies developed either from individuals seeking a change in food or as a result of the negative geotropism of the newly hatched crawler.

The evidence also is that they are temporary, either as a result of movement downward or their elimination by some biological control factor. The usual predators on mealybugs, coccinellids, et cetera, appear on pineapple only when very large colonies have established, and the only other possible predator noted in the field was an undetermined species of spider which builds a frail web in the center of the plant.

There was ample evidence, however, of the temporary and discontinuous presence of small mealybug colonies on the leaves.

Incidental Wilted Plants.—These occur in pineapple fields usually when the plants are differentiated or in the early blossom stage. Exact diagnosis is complicated by the absence of a typical ant-mealybug association and by the fact that the loss of roots from other causes can result in a "wilted" plant with symptoms very similar to those of typical mealybug wilt.

Three counts were made of incidental wilts during the course of the experiment and in each case the entire field was inspected plant by plant and the wilted plants staked. The first of these counts was made in July 1955 when the plants were 9 months old; the second at the time of differentiation in November-December; and the third when the majority of the plants were in the green fruit stage, the following March.

These three counts are summarized in table 1 and the actual locations mapped in figure 2. This mapping was made from the air, but the aluminum sheets placed over the stakes showed up too indistinctly for reproduction so pins were inserted in the enlarged print. These pins fix the locations of the wilted plants exactly, but the size of the pin exaggerates the area involved.

Table 1 shows that the time of onset of these incidental wilts followed the general pattern, that is rare in young plants, noticeable in plants at differentiation, with full development reached in the early fruiting stage.

When sprayed and unsprayed blocks are compared, it will be seen that the number per acre varies considerably

Table 1.—Incidence of scattered wilted plants.

Block	Area, Acres	Treatment[a]	July 1955	Nov.–Dec. 1955	March 1956	Wilted Plants	
						Total	Per Acre
8	1.8	U	4	5	27	36	20.1
9	2.1	S	1	5	44	50	23.8
10	2.0	U	0	13	38	51	25.0
11	2.0	S	2	11	27	40	20.0
12	2.0	S	0	13	10	23	11.9
13	2.4	U	0	10	42	52	21.1
14	2.5	U	1	41	59	101	41.1
15	2.1	S	2	11	71	84	40.8
16	1.7	S	0	4	31	35	20.0
17	1.7	U	0	12	23	35	20.0
18	1.6	U	2	7	47	56	34.8
19	1.7	S	—	23	35	58	34.1
Totals	11.6	S	5	67	218	290	25.1
Totals	12.1	U	7	88	236	331	27.4

[a] S = Sprayed; U = Unsprayed.

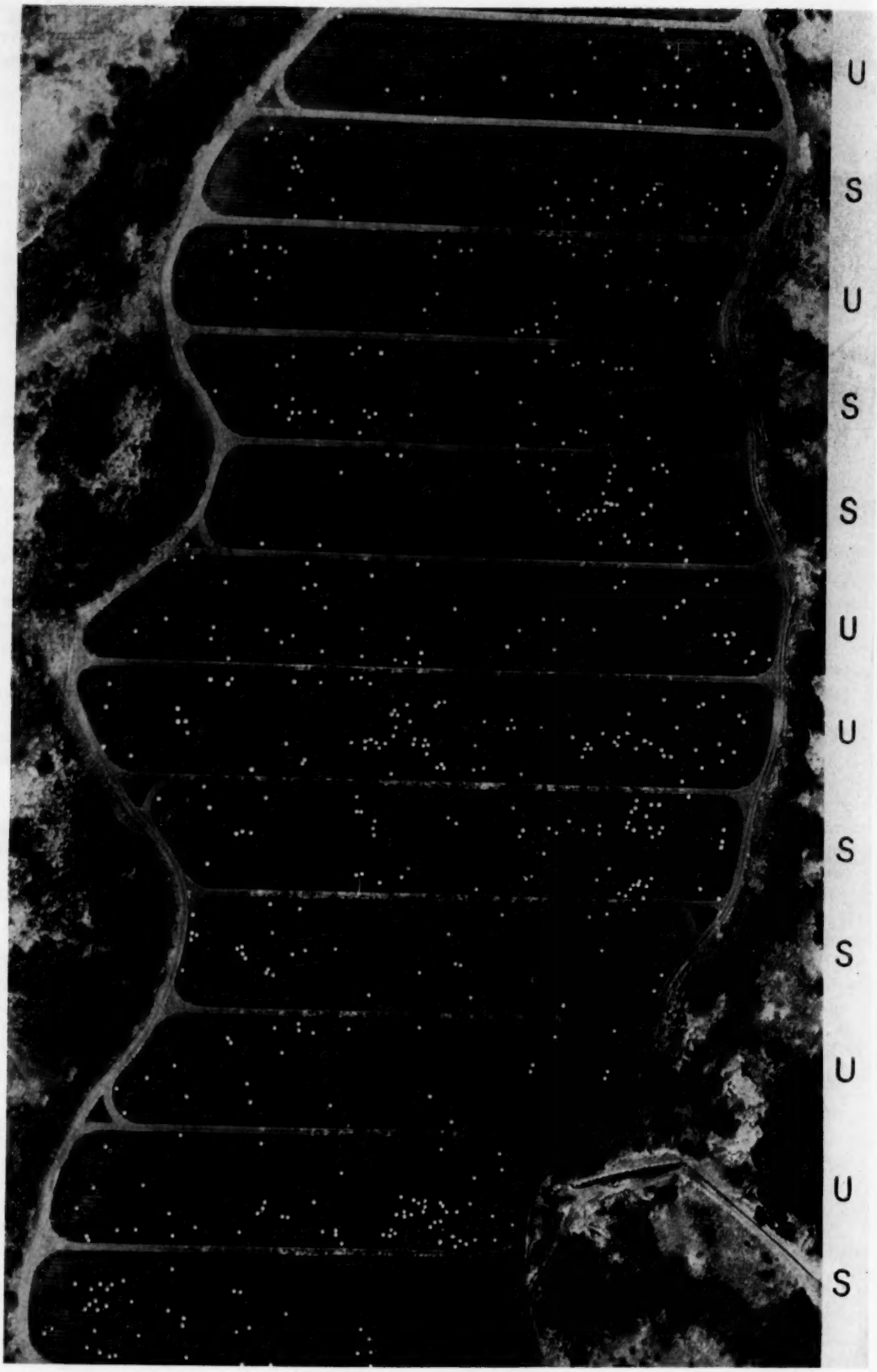

FIG. 2.—Aerial photograph of the experimental field showing the location of scattered wilted plants. The dark lines conspicuous in the upper part of the picture show the location of old roads in the previous cycle. S=Sprayed; U=Unsprayed.

Table 2.—Summary of mealybug counts on roots and stumps of plants sampled before harvest.

Block[a]	Per Cent Plants Infested		Average No. Mealybugs per Plant	
	Healthy Area	Wilted Area	Healthy Area	Wilted Area
8 U	60	100	1.8	11.5
9 S	50	100	4.2	16.8
10 U	30	100	1.7	10.6
11 S	20	100	2.7	10.3
12 S	40	67	4.9	8.3
13 U	20	100	.6	7.0
14 U	35	100	8.8	1.5
15 S	60	100	2.9	11.7
16 S	0	100	0	18.4
17 U	20	100	1.1	12.0
18 U	40	50	7.4	4.2
19 S	30	100	2.4	43.0

[a] S = Sprayed; U = Unsprayed.

with location in the field but with no relationship ascribable to spray treatment. It is also clear that many small areas show higher concentrations than the average.

Mealybug Counts.—Counts were made before harvest by pulling plants up and making a total count by dissection of the plant and the root mass. One series was pulled from areas showing no incidental wilts; the other from areas where the wilted plants were most numerous. The results of these counts are indicated in table 2 and show a significant difference in per cent plants infested and in average number of mealybugs per plant between the two areas in each block but again with no important difference between the sprayed and unsprayed blocks. Block 14 is the only exception, and this may be related to the earlier appearance of many of the incidental wilts in that block.

Discussion.—The rough correlation between numbers and size of mealybug colonies and areas showing incidental wilts might be considered adequate to associate incidental wilting with the occasional leaf feeding by subterranean mealybug colonies. Critical examination of the aerial photograph, however, will reveal dark streaks representing better growth of the plants in old roadways. When the number of incidental wilts in these old roadways is compared with the field in general, there is a definite reduction noted in the old roadways. Taking the first six blocks where the old roads are better defined, the values are 0.78 vs. 1.38 per pineapple bed for old roadway and field, respectively, a difference which is significant. Since the improved growth often seen in old roadways when these are ploughed up and included in a new field layout is ascribable to a partial soil-sterilizing effect, the role of mealybugs in incidental wilting might well be in the accumulative establishment of courts of entry for weak root pathogens. The only typical mealybug wilt occurring in this field was in two small spots on the field margin where ants became established.

Screening Tests with Synthetic Compounds as Synergists for House Fly Sprays[1]

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ABSTRACT

Fifty-six synthetic compounds were tested in space sprays by the Campbell turntable method against house flies, *Musca domestica* L., as synergists with pyrethrins or allethrin. The most effective compounds were the butyl 2-methyl-3-(3,4-methylenedioxyphenyl) propyl acetal of acetaldehyde and its isobutyl analog.

Some of the chemicals synthesized at the Beltsville, Maryland, laboratory (Beroza 1956, Alexander & Barthel 1957, Barthel & Alexander 1957, Piquett *et al.* 1958) have been tested as synergists for pyrethrins and allethrin. The screening procedure was similar to that used previously (Piquett *et al.* 1958). This paper reports the results of these tests.

MATERIALS AND METHODS.—Sprays were prepared to contain 0.5 mg. of pyrethrins or 0.25 mg. of allethrin per milliliter plus a tenfold concentration of the test adjunct. The solvent was usually refined kerosene (Deobase), but with some materials acetone had to be added for solubility purposes. Similar mixtures were prepared with piperonyl butoxide as the adjunct.

The sprays were applied by the Campbell turntable method to house flies (*Musca domestica* L.) in duplicate tests. Approximately 100 adult flies, 2 to 3 days old, were used in each test. For comparison standard sprays containing insecticides only—1, 2, and 4 mg. of pyrethrins and 0.5, 1, and 2 mg. of allethrin per milliliter—were also tested against each population of flies. In a given series the same solvent was used in the standard sprays as in the mixtures.

RESULTS.—The sprays caused complete or nearly complete knockdown; the mean mortalities obtained with those that showed any synergistic effect are given in table 1. To avoid reporting the results in many series because of the use of many populations of flies, the following procedure was adopted. The mortalities obtained for the standards in a series, consisting of the tests on the same populations of flies, were plotted on log probability paper and the straight line for each insecticide was estimated graphically. Previous work with but two replications had indicated that 1.3 mg. of pyrethrins per milliliter and 0.67 mg. of allethrin were the minimum concentrations required to cause mortalities significantly higher than those caused by the concentrations used in the mixtures. The mortalities at these concentrations were determined from the straight lines. Since the adjuncts were not toxic or only negligibly so (Piquett & Gersdorff 1958, 1959), a

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Table 1.—Per cent mortalities of house flies caused by sprays containing pyrethrins or allethrin in refined kerosene with and without adjuncts.

Adjunct	Pyrethrins		Allethrin	
	Mixture (0.5+5.0 mg./ml.)	Alone (1.3 mg./ml.)	Mixture (0.25+2.5 mg./ml.)	Alone (0.67 mg./ml.)
Very strong synergism with pyrethrins				
Acetaldehyde, butyl 2-methyl-3-(3,4-methylenedioxyphenyl)propyl acetal	98	23	59	30
Acetaldehyde, isobutyl 2-methyl-3-(3,4-methylenedioxyphenyl)propyl acetal	95	15	38	26
Piperonyl butoxide	98	34	50	48
Strong synergism with pyrethrins				
Acetaldehyde, 2-(2-ethoxyethoxy)ethyl 3-(3,4-methylene dioxyphenyl)propyl acetal	89	36	43	28
Acetaldehyde, 2-(2-ethoxyethoxy)ethyl α-methylpiperonyl acetal[a]	58	13	88	68
Acrylamide, *N,N*-diethyl-3-(3,4-methylenedioxyphenyl)-2-phenyl-[a]	81	54	47	65
Acrylic acid, 3-(3,4-methylenedioxyphenyl)-2-phenyl-, ethyl ester[a]	55	46	68	81
Acrylic acid, 3-(3,4-methylenedioxyphenyl)-2-phenyl-, methyl ester[a]	85	46	83	81
Pyran, tetrahydro-2-(2-allyl-4,5-methylenedioxyphenoxy)-	74	23	26	30
Pyran, tetrahydro-2-piperonyloxy-	50	16	37	43
Borderline to moderate synergism with pyrethrins				
Acetaldehyde, isobutyl 6-propylpiperonyl acetal	27	23	30	30
5-Hexenoic acid, 6-(3,4-methylenedioxyphenyl)-4-oxo-, ethyl ester	18	16	19	43
Piperonal, diethyl acetal	17	16	14	43
Propionic acid, 3-(3,4-methylenedioxyphenyl)-2-phenyl-, ethyl ester	35	36	6	28
Propionic acid, 3-(3,4-methylenedioxyphenyl)-2-phenyl-, methyl ester	42	36	2	28
Tropic acid, 3-(3,4-methylenedioxyphenyl)-, ethyl ester, acetate	33	53	52	58
Tropic acid, 3-(3,4-methylenedioxyphenyl)-, methyl ester, propionate	60	53	58	58
Tropic acid, 3-(3,4-methylenedioxyphenyl)-, methyl ester, acetate	34	37	44	39

[a] Solvent 1:3 acetone-kerosene.

mortality equal to or higher than that caused by these concentrations indicated synergistic action.

Two compounds, butyl 2-methyl-3-(3,4-methylenedioxyphenyl)propyl acetal of acetaldehyde and its isobutyl analog, were very effective synergists for pyrethrins, perhaps as effective as piperonyl butoxide. However, more extensive testing is required for a conclusive comparison. Seven other compounds showed strong effect with pyrethrins and eight showed borderline to moderate synergism. In general, those compounds found synergistic with pyrethrins had similar action with allethrin; however, the effect with allethrin, as in a mixture containing one of our best commercial synergists, was never more than moderate.

The following compounds showed no synergism with either insecticide. Very few of these contained the methylenedioxyphenyl group, whereas all the effective compounds contain this group.

Acetaldehyde,
2-(2-butoxyethoxy)ethyl 2-(ethoxyethoxy)ethyl acetal
2-(2-butoxyethoxy)ethyl isobutyl acetal
butyl heptyl acetal
butyl *p*-methoxybenzyl acetal
butyl l-methylheptyl acetal
2-(2-ethoxyethoxy)ethyl heptyl acetal
2-(2-ethoxyethoxy)ethyl *p*-methoxybenzyl acetal
2-(2-ethoxyethoxy) ethyl 3-methoxybutyl acetal
2-(2-ethoxyethoxy)ethyl l-methylheptyl acetal
ethyl phenyl acetal
isobutyl heptyl acetal
isobutyl *p*-methoxybenzyl acetal
isobutyl *m*-methylbenzyl acetal
isobutyl l-methylheptyl acetal
isobutyl phenyl acetal
phenyl-, diethyl acetal
Acrylamide, *N,N*-diethyl-3-methoxyphenyl-2-phenyl
Acrylic acid,
3-(*o*-methoxyphenyl)-2-phenyl-, methyl ester
3-(*p*-methoxyphenyl)-2-phenyl-, ethyl ester
3-(*p*-methoxyphenyl)-2-phenyl-, methyl ester
o-Anisaldehyde, diethyl acetal
p-Anisaldehyde, diethyl acetal
Benzaldehyde,
o-(allyloxy)-, diethyl acetal
p-isopropyl-, diethyl acetal
o-(2-methylallyloxy)-, diethyl acetal
Chrysanthemumic acid,
p-allyloxybenzyl ester
2-allyl-4,5-methylenedioxyphenyl ester
6-nitropiperonyl ester
Cinnamaldehyde, diethyl acetal
3-Cyclohexene-1-carboxaldehyde,
diethyl acetal
l-methyl-, diethyl acetal
Piperitone
Propionaldehyde, 2,2,3-trichloro-, diethyl acetal
Propionic acid,
3-(*o*-methoxyphenyl)-2-phenyl-, methyl ester
3-(*p*-methoxyphenyl)-2-phenyl-, ethyl ester
3-(*p*-methoxyphenyl)-2-phenyl-, methyl ester
3-(3,4-methylenedioxyphenyl)-, ethyl ester
3-(3,4-methylenedioxyphenyl)-, methyl ester
Veratraldehyde, diethyl acetal

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Methods for the Evaluation of Stable Fly Toxicants and Repellents[1]

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ABSTRACT

The spot test consists in applying materials to small areas on cattle and confining stable flies (*Stomoxys calcitrans* (L.)) on these areas at various intervals after treatment. Materials that are effective in this test are further evaluated by treating an entire animal and placing it in a screened cage with flies. Results of spot tests with several insecticides and a comparison of results obtained in tests with methoxychlor by both methods are presented.

Each year thousands of new compounds are synthesized as potential insecticides. The expense involved in their production limits the amounts that are made available for testing, and any one laboratory may have as little as 2 grams with which to work. Efficient test methods are necessary to utilize these limited amounts to the greatest advantage.

At the Kerrville, Texas, laboratory two methods have been devised for the preliminary evaluation of insecticides. The stable fly (*Stomoxys calcitrans* (L.)) is used as the test insect because it is easy to rear and work with under laboratory conditions (McGregor & Dreiss 1955), and because it is an important pest in many sections of the United States.

SPOT TESTS.—A spot-test technique has been developed that permits an initial evaluation with 250 mg. or less of a compound and in one application a compound can be evaluated as a toxicant, a knockdown agent, and a repellent.

This method consists in applying the materials to small areas on a Hereford steer or cow. Areas 6 inches in diameter are marked with black dye on the upper half of the body in such a manner that no area is directly below another and each is on a fairly smooth spot (fig. 1). On a large animal six of these areas can be grouped on a side, and on smaller animals five can be adequately spaced. During the winter the hair on each area is clipped to $\frac{1}{4}$ to $\frac{1}{2}$ inch in length, so that the flies can reach the skin to feed, but clipping is not necessary when the animals are in summer coat.

The test material is dissolved in acetone, or in another solvent when insoluble in acetone, and applied with a De Vilbiss atomizer using compressed air as a propellent. The spray is confined to each area with a metal cone (fig. 2).

FIG. 1.—Hereford heifer with five test areas, three with testing cages in position. Muzzle prevents animal from licking the treated area.

Any concentration may be used, but 5% is ordinarily used for the initial test. The volume used was determined from the holding capacity of the hair, and since 5 ml. gave adequate coverage of the test area with no run-off, it is the standard volume used for this test.

Cages are made from Mason-jar lids by soldering a piece of screen wire in place of the removable top. The cages are $3\frac{1}{2}$ inches in diameter, $\frac{1}{2}$ inch deep at the edge, and $\frac{3}{4}$ inch deep at the center. Each cage is supplied with a metal tray. The cage is held to the tray by a flanged edge, which is slid into the rolled edge of the tray (fig. 3).

Stable flies 3 to 6 days old that have not fed for 18 hours are anesthetized with carbon dioxide, and 25 flies are placed in each cage (fig. 4). When the flies recover from the anesthetic, the cage is slipped off the tray onto the treated area and the flies are exposed for 20 minutes. It was found that a period of at least 15 minutes is required for 90% or more of the flies feeding on untreated areas to engorge. The cage is held in position with two pairs of clips, joined with a heavy rubber band (figs. 5, 6), which are anchored to hair treated with rubberized branding cement at four points around the treated area. The rubber bands, under tension, hold the cages in position regardless

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FIG. 2.—Treating a test spot using the metal cone to prevent cross contamination.

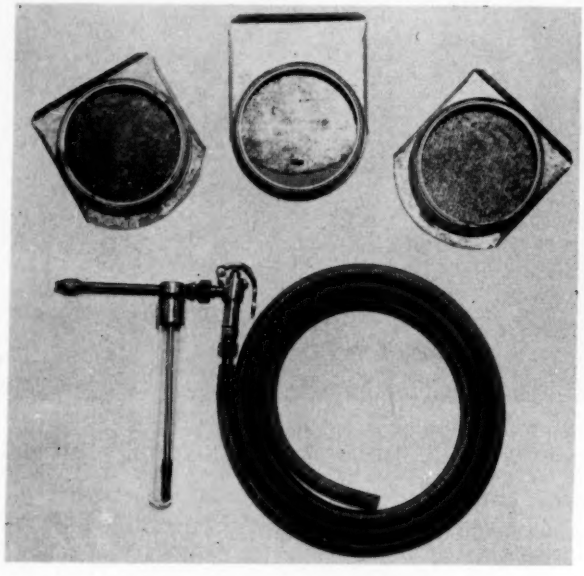

FIG. 3.—Test cages with attached trays and the atomizer used for application.

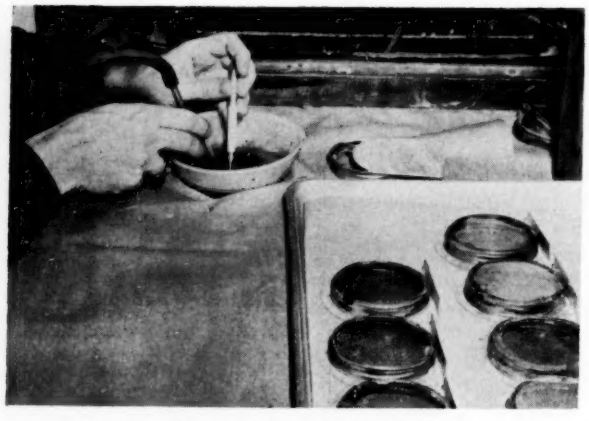

FIG. 4.—Placing CO_2-anesthetized stable flies into individual cages.

FIG. 5.—Exposing stable flies to a treated area.

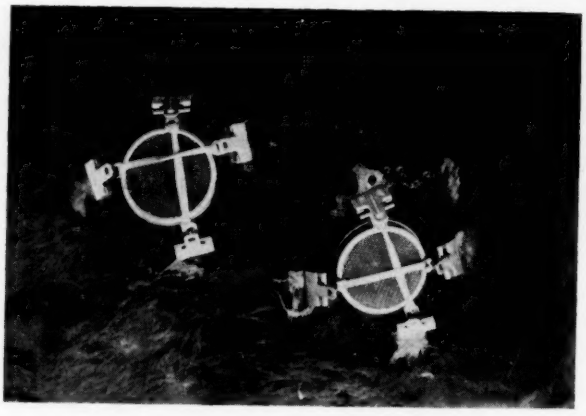

FIG. 6.—Closeup of cages on treated areas.

of the animal's movements. After exposure the flies are moved to a constant-temperature room for holding at 72° F. and 75% relative humidity.

Toxicity is measured by the per cent mortality of the flies 24 hours after exposure, and knockdown by the per cent of the flies unable to fly or walk immediately after they are removed from the test area. Repellency is measured by subtracting the per cent of flies that fed from 100%. When any of the values fall below 90%, the material is considered as having failed for that effect. The test is discontinued only after the material fails in all three respects.

CAGE TESTS.—Materials showing promise in spot tests are further evaluated in cage tests. A Jersey yearling is sprayed with 1 gallon of the insecticide at a concentration indicated by preliminary data to be effective without poisoning the animal. On different days after treatment the animal is placed in a screened cage and exposed to 100 starved flies. Two hours after their release all flies remaining alive are collected, the number that fed are recorded, and the flies are held 24 hours for mortality counts. The difference between the number released and the number collected is used as a measure of knockdown. This difference plus the number dead after 24 hours gives the per cent control. When this procedure is used on untreated animals, 90% to 100% of the released flies are

collected, with an average of 90% feeding and 10% mortality.

RESULTS OF TESTS BY TWO METHODS.—In order to evaluate the usefulness of these test methods, a number of insecticides that had been field-tested for stable fly control were used in spot tests and methoxychlor was used in a cage test. For the spot tests they were applied in acetone solutions at 0.5%, except for pyrethrum which was applied as 0.05% of pyrethrins. For the cage test a 0.5% suspension of methoxychlor was used.

The data shown in table 1 indicate that the spot test can be expected to give a fair indication of the residual effectiveness and repellency of an insecticide, as the results obtained with methoxychlor, malathion, toxaphene, and pyrethrum compared with each other in much the same way as results of usual field applications. All the compounds, however, are more effective in spot tests than when used under practical conditions.

It is interesting to compare the results obtained with methoxychlor in the two types of tests (tables 1 and 2). Effectiveness at the 100% level is not included in table 1, but methoxychlor caused 100% mortality and knockdown for 7 days. In the cage tests 100% mortality did not occur after 4 days and 100% knockdown was not achieved at any of the test intervals. One reason for this difference is that when the whole animal is treated some areas lose the insecticide more rapidly than others. These losses may be due to licking, rub-off, switching of the tail, absorption through the skin, and other causes. As the decontaminated areas become larger, more flies feed on them. However, there is a question as to whether the flies feed at random or seek areas with little or no insecticide.

The spot-test data indicate that methoxychlor acts as a repellent for 2 days. However, the repellency is strongly influenced by the speed of knockdown. During the first 2 days flies were affected so rapidly they had little opportunity to feed. Since flies were not exposed in the cage test until 4 days after treatment, a 90+% repellency was not observed.

To determine the effect of using different formulations for the two tests, a spot test was conducted with a 0.5% suspension of methoxychlor. There was little difference between the results of this test and those obtained with the acetone solution. The effective period was 9 days for mortality and 8 days for knockdown, as compared with 9 and 7 for the solution. No repellency was noted because of the slower knockdown action of the wettable powder formulation.

The spot test can be used in testing both single compounds and combinations. There is evidence that it is a valuable tool in studying the chemical and physical action of insecticides on the hair of cattle. It can also be adapted to bioassay procedures using organisms other than stable flies.

The cage test is an important step in secondary evaluation, since it more closely resembles practical application than the spot test. An entire animal is treated, and flies are free to feed on any part of the body. In addition, the insecticide is exposed to many of the same factors, such as sunlight, rain, mud, and abrasion, that apparently cause loss of effectiveness in the field.

Table 1.—Days that response of six insecticides was 90% or greater against stable flies by the spot-test method of evaluation.

INSECTICIDE	MORTALITY	KNOCKDOWN	REPELLENCY
DDT	7	4	0
Methoxychlor	9	7	2
Malathion	1	0	0
Toxaphene	5	0	0
Co-Ral[a]	10	0	0
Pyrethrum	2	2	3

[a] *O*-(3-chloro-4-methylumbelliferone) *O,O*diethyl phosphorothioate. Also designated Bayer 21/199.

Table 2.—Duration of effectiveness of a 0.5% suspension of methoxychlor against stable flies collected 2 hours after release of 100 flies on a treated animal in a cage.

DAYS AFTER TREATMENT	NUMBER			PER CENT CONTROL
	Collected	Fed	Dead After 24 Hours	
4	17	10	17	100
6	23	14	12	89
8	65	59	13	48
11	72	71	8	6

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PACIFIC BRANCH MEETING
ENTOMOLOGICAL SOCIETY OF AMERICA

June 28, 29, 30, 1960

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Spokane, Washington

Effect of Parathion on Parasites of Oriental Fruit Moth Cocoons and *Trichogramma minutum* in Peach Orchards[1]

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Abstract

In peach orchards near Moorestown, New Jersey, cocoon parasitism of the oriental fruit moth (*Grapholitha molesta* (Busck)) was about 86% lower, fewer species of parasites were in evidence, and parasitization of eggs by *Trichogramma minutum* Riley occurred in 20% fewer exposure periods in orchards sprayed in early spring with parathion than in orchards sprayed with lead arsenate. In orchards sprayed with parathion, cocoon parasitism was comparatively low through most of the growing season. In one of the 2 years these studies were conducted, *T. minutum* moved rapidly into orchards soon after the last application of parathion.

The toxicity of insecticides to parasites of the larvae of the oriental fruit moth (*Grapholitha molesta* (Busck)) has been reported by many authors (Allen 1957, 1958, Fleming *et al.* 1951, Peterson 1946, Pielou & Glasser 1951, and Smith & Driggers 1944). However, little information has been published on their toxicity to the cocoon and egg parasites of this pest. In New Jersey parathion, 4 ounces per 100 gallons, is commonly applied to peach orchards at shuck split and 10 and 20 days afterward to control this moth and the plum curculio (*Conotrachelus nenuphar* (Hbst.)) simultaneously. In some orchards, however, lead arsenate, 2 pounds, plus hydrated lime, 16 pounds, is applied at shuck split and 10 days later to control plum curculio. This insecticide has little effect on the oriental fruit moth or its parasites. In both programs wettable sulfur, 4 or 5 pounds per 100 gallons, is included for disease control.

In 1956 and 1957 the effect of early applications of parathion on oriental fruit moth cocoon parasites and on *Trichogramma minutum* Riley, one of its egg parasites, was studied in peach orchards in the vicinity of Moorestown, New Jersey. The objectives were to ascertain the species of parasites present and the comparative degrees of cocoon and egg parasitism in June, July, and August in orchards sprayed with parathion or lead arsenate. Observations were made each year in four orchards sprayed with each insecticide. The first sprays were applied about May 16 in 1956 and May 6 in 1957. No insecticides were applied after the first week of June, but additional applications of sulfur were made in most of them.

Rainfall at Moorestown during these observations varied considerably and may have affected the results. In 1956 rainfall from April through August was about normal and totaled 18.54 inches. In 1957 it was about normal in April and much below normal during the next 4 months; the total for the 5-month period was 10.66 inches below normal.

Effect on Cocoon Parasites.—Oriental fruit moth cocoons are difficult to find on trees and in rubbish under trees. Therefore, information on cocoon parasites was obtained by pinning, with straight pins about three-fourths inch long, one cocoon in corrugated cardboard to the trunk and one to each of the large branches, usually four, about 8 to 10 inches from the trunks of 25 trees in each orchard. These marked trees were usually four trees apart in each fourth row.

Exposure of cocoons was continuous from May 25 to August 15 in 1956 and from May 15 to August 23 in 1957. Early in the season cocoons were exposed for 10 days, but as the days became warmer the period was reduced to 7 days to prevent emergence of moths or parasites. Exposed cocoons were replaced with fresh cocoons in half the orchard during 1 day and in the other half 2 days later. This resulted in some overlapping of exposure periods. As soon as the cocoons were removed from the orchards, they were isolated in shell vials plugged with cotton for insect emergence.

The cocoons were produced in the laboratory on small thinning-size apples, about 1 inch in diameter, at 80° F. Cocoons were formed in corrugated cardboard strips that were removed daily from breeding trays, but were not placed in orchards until the following day.

A total of 9,985 cocoons were exposed to parasitization in 1956 and 14,000 in 1957. Although some were lost to birds, mice, and ants, moths or parasites emerged from 70% of the cocoons exposed each year.

The percentage of cocoon parasitization during each exposure period is shown in table 1, and the species of parasites reared in table 2. Parasitization was about 86% lower in orchards sprayed with parathion than in those sprayed with lead arsenate. In the parathion-sprayed orchards parasitization remained at a comparatively low level throughout the growing season, except in August 1956.

The slowness with which the parasites repopulated the orchards was in sharp contrast to the reported activity of other parasites of the oriental fruit moth. Allen (1958) observed that species that parasitize larvae in succulent peach twigs usually reappeared in normal numbers about 3 weeks after DDT was applied. Apparently *Macrocentrus ancylivorus* Roh., the predominant species reared in his studies, moves widely in search of its host, thereby replacing rapidly the population deficit caused by application of compounds toxic to parasites. The number of species reared is evidence that most parasites of oriental fruit moth cocoons disperse slowly and probably make infrequent flights. Only five species were obtained from cocoons exposed in orchards sprayed with parathion in 1956 and four in 1957, whereas 10 and 15 species, respectively, were reared from the orchards sprayed with lead arsenate. It is possible that parathion affected most the species that overwinter in oriental fruit moth cocoons, since *Eurytoma appendigaster* (Swed.), *Eupelmus cyaniceps cyaniceps* Ash., *Eupelmus limneriae* How., *Mastrus pilifrons* (Prov.), and *Arachnophaga frontalis* Gah. were not reared, except in one case, from cocoons placed in orchards sprayed with parathion. Furthermore, no hibernating parasite larvae were found in cocoons exposed

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[2] E. J. Wilkins, E. L. Plasket, and Richard Smythe, biological aids, assisted in these studies.

Table 1.—Percentage of oriental fruit moth cocoons parasitized in orchards sprayed with parathion or lead arsenate.

EXPOSURE DATES	PARATHION	LEAD ARSENATE
1956		
May 25–June 8	0	8.8
June 5–18	0	17.8
15–27	0.3	23.3
22–July 4	.3	25.9
29–July 11	0	19.0
July 6–18	1.7	23.3
13–25	2.5	16.7
20–Aug. 1	2.3	16.8
27–Aug. 8	1.1	12.5
Aug. 3–15	19.0	17.7
All season	2.5	18.0
1957		
May 15–24	0.5	2.4
22–31	.2	6.3
29–June 7	.5	8.1
June 5–14	5.4	14.9
12–21	1.0	21.9
19–28	1.2	16.2
26–July 5	0	13.5
July 3–12	1.3	9.0
10–19	0.3	3.6
17–26	.5	1.9
24–Aug. 2	2.6	2.0
31–Aug. 9	0.9	3.3
Aug. 7–16	1.7	5.7
14–23	2.7	5.5
All season	1.1	8.3

in parathion-sprayed orchards. It is possible that the increase of parasitism in August resulted from the migration of some species. Periodic migration of *Calliephialtes grapholithae* (Cress.), *Coccygomimus inflatus* (Tow.), and *Itoplectis conquisitor* (Say) is suspected, since they were reared chiefly from cocoons exposed in August.

The degree of parasitization observed in these studies does not indicate the total value of cocoon parasites in suppressing the oriental fruit moth in orchards under natural conditions and in orchards sprayed with compounds, such as lead arsenate, of low toxicity to parasites. The cocoon period for transforming individuals averages 14 days in New Jersey (Peterson & Haeussler 1930), which is longer than cocoons were exposed to parasitization in these studies. If the cocoons could have been exposed for their full term, parasitism would probably have been considerably greater. Brunson & Allen (1948) reported that cocoon parasitism averaged 15% in eight eastern States and frequently reached 50% in individual orchards. Driggers (1932) reported an average of 66% cocoon parasitism in 1928 and 30% in 1929.

Effect on Trichogramma minutum.—Oriental fruit moth eggs for parasitization by *Trichogramma minutum* were obtained in the laboratory by placing peach leaves, one per twig in water, with moths in a small screen-wire cage. They were exposed to parasitization for 2 days each week from May 22 to August 9 in 1956 and from May 14 to August 15 in 1957. A twig bearing one leaf containing about 50 eggs deposited the preceding night was placed in a small shell vial containing water, which was plugged with cotton covered with a thin plastic film. The vials were then attached with rubber bands to spring-type clothespins, which were clipped to branches near the top

Table 2.—Percentage of different species in total parasitization of oriental fruit moth cocoons exposed in orchards sprayed with parathion or lead arsenate.

PARASITE	1956		1957	
	Parathion	Lead Arsenate	Parathion	Lead Arsenate
Arachnophaga frontalis Gah.	—	2.8	3.8	5.1
Calliephialtes grapholithae (Cress.)	20.0	—	—	—
Coccygomimus inflatus (Tow.)	23.7	39.1	53.8	35.6
Dibrachys cavus (Walk.)	18.7	6.5	40.4	10.5
Dibrachys sp.	—	.2	—	—
Eupelmus cyaniceps cyaniceps Ash.	—	.4	—	—
Eupelmus limneriae How.	—	5.2	—	7.6
Eupelmus sp.	—	—	1.9	.4
Eurytoma appendigaster (Swed.)	—	13.8	—	14.2
Gambrus ultimus (Cress.)	5.0	1.9	—	—
Gelis tenellus (Say)	—	.9	—	.4
Gelis sp.	—	.2	—	—
Haltichella xanticles (Walk.)	—	.6	—	—
Itoplectis conquisitor (Say)	32.5	—	—	—
Lymeon orbum (Say)	—	9.5	—	2.1
Mastrus pilifrons (Prov.)	—	2.1	—	5.8
Phaeogenes walshiae walshiae Ashm.	—	.2	—	5.5
Tritneptis hereocompae Grlt.	—	.2	—	—
Hibernating parasites	—	16.3	—	12.7

of trees on which cocoons were exposed. The exposed leaves were placed in contact with leaves of the tree to increase the possibility of parasitization. One leaf was exposed each week in each of 5 trees per orchard in 1956 and 10 trees in 1957. Each successive week exposures were made in the next 5 or 10 of the 25 trees marked for use in each orchard, and so on throughout the orchard several times during the season. Exposed leaves were held in the laboratory until unparasitized eggs hatched to determine the presence of *T. minutum*.

Observations on the effect of parathion and lead arsenate on egg parasitization by *T. minutum* are shown in

Table 3.—Number of orchards sprayed with parathion or lead arsenate in which oriental fruit moth eggs were parasitized by *Trichogramma minutum*.[a]

EXPOSURE DATES	PARATHION	LEAD ARSENATE
1956		
May 22–24	2	3
29–31	0	2
June 5–7	0	0
12–14	0	1
19–21	0	3
26–28	0	0
July 3–5	1	2
10–12	0	2
17–19	0	3
24–26	3	3
31–Aug. 2	4	1
Aug. 7–9	4	4
1957		
May 14–16	1	3
21–23	0	1
28–30	0	1
June 4–6	1	2
11–13	2	3
18–20	2	4
25–27	2	4
July 2–4	4	1
9–11	3	3
16–18	3	4
23–25	3	4
30–Aug. 1	4	4
Aug. 6–8	3	4
13–15	4	4

[a] Four orchards sprayed with each insecticide.

table 3. The difference in parasitism between the 2 years apparently is characteristic of the species. Many authors have reported marked annual differences, that the parasite often becomes most abundant during the latter part of the growing season, and that parasitism varies considerably in adjacent orchards or areas. Egg parasitization occurred in 29% of the exposure periods in orchards sprayed with parathion and in 50% in those orchards sprayed with lead arsenate in 1956, and 57% and 75%, respectively, in 1957. There was only a slight and inconsistent difference between the total number of exposures in which parasitization occurred each year in the orchards sprayed with the insecticides. The *T. minutum* population was affected by parathion, but in 1957 the parasite moved into the orchards rather rapidly after the last application. Results obtained in 1956 suggested repopulation of the affected area at a slow rate. However, this condition may have resulted from a scarcity of the parasite in the area around the orchards, as Schread & Garman (1933) and Jaynes & Bynum (1941) reported this parasite capable of dispersing over a considerable area in 2 to 3 days. On leaves on which parasitization of eggs occurred 42% were parasitized in 1956 and 44% in 1957.

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Residues in Established Alfalfa Treated with Granulated Phorate (Thimet) and Their Effect on Cattle Fed the Hay[1]

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ABSTRACT

Granulated phorate (formerly designated as Thimet) at 1 and 4 pounds actual per acre was applied to each of two plots of established alfalfa immediately following cutting and prior to an irrigation. Hay from these two plots harvested 27 days after treatment, was fed to two groups of five steers each for a 1-month period, while a similar group was fed hay from an untreated plot. All groups received untreated hay for a second month. Cattle fed hay from the 4-pound treatment gained weight at a lesser rate than cattle fed hay from the 1-pound treatment, and these in turn gained at a lesser rate than cattle fed untreated hay. The differences, however, were not significant. These differences were smaller during the second month when only untreated hay was fed to the three groups; however, the reduced number of individuals does not allow a valid comparison for this period.

Residue determinations using an electrometric technique involving inhibition of blood plasma cholines terase were made on alfalfa samples taken during the growth period following treatment. Maximum residue concentrations, 390 ± 236 and 520 ± 225 parts per million for the lower and higher treatment rates, respectively, were found on the third day after treatment. Samples from the 1-pound plot showed no residues on the 14th day, while those from the 4-pound rate continued to show residues through the 21st and perhaps through the 25th day. No residues were found in air-dried hay from the 4-pound plot harvested on the 27th day.

Cholinesterase activity of blood samples taken periodically from the steers during the feeding period was determined by a spectrophotometric method. Considerable variation in this activity was found between animals within groups and furthermore between sampling dates on individual animals. No evidence was found to indicate that blood cholinesterase activity was appreciably affected by the ingestion of hay from either of the two treatments.

Systemic insecticides for the control of spotted alfalfa aphids (*Therioaphis maculata* (Buckton)) on established stands of alfalfa, tested in New Mexico for 3 years (Dobson 1958), have shown good control under the conditions of application. If no residues of these chemicals remain in the hay at cutting time, and if there are no deleterious effects upon animals fed the hay, these materials might prove acceptable for commercial use. Because of the interest in these materials and their potential use, an experiment with granulated phorate (formerly designated as Thimet) was instituted to gain information on the follow-

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ing points: (1) persistence of residues in the plants through the harvest stage; (2) possible effects on weight gains of cattle fed hay from treated alfalfa; and (3) possible effect on cholinesterase levels in blood from the same cattle.

Residual persistence of phorate and its metabolites has been determined in small grains (Lilly *et al.* 1958) and in a number of vegetable crops (Bowman & Casida 1957) by the blood cholinesterase inhibition method. The metabolism of phorate in plants through a series of principally enzymatic oxidations to form a number of compounds possessing greater anticholinesterase activity than phorate itself has been extensively studied by Bowman & Casida (1957, 1958) and by Metcalf *et al.* (1957).

Determination of cholinesterase activity of the erythrocyte fraction of bovine blood by an electrometric method has been reported by Hermenze & Goodwin (1959). Petty & Lovell (1958) also used an erythrocyte fraction but employed a colorimetric technique. Robbins *et al.* (1958) have reported results using whole blood with the colorimetric method. These authors report that the major share of the cholinesterase activity is located in the red blood cells, the plasma fraction contributing, on the average, less than 5% of the total activity.

MATERIALS AND METHODS.—*Field Procedure.*—A third-year field of New Mexico 11-1 alfalfa which had two current season cuttings was selected for treatment. Three plots were set out, each consisting of 2 acres. On June 19, 1958, before growth had resumed for the third cutting, phorate as 2% granulated was applied at the rate of 1 and 4 pounds actual per acre to each of two plots. The lesser rate was that previously found adequate for aphid control (Dobson 1958), while the higher rate was that suggested as a trial safety factor by the Pure Food and Drug Administration. The material was applied with a 4-foot Gandy fertilizer spreader. The third plot was held as an untreated check. All three plots were flood irrigated within 6 hours after application was completed.

Samples of green alfalfa for residue analyses were taken 1, 3, 5, 7, 14, 21, and 25 days after application and held frozen until analyses were made. Hay was taken at random from five places in each plot and pooled to make a composite sample of approximately 2 pounds of green material per plot. The plots were cut on the 27th day after treatment and the hay was sun-dried and baled in the conventional manner. The bales were stored in a barn for the feeding portion of the experiment.

PHORATE RESIDUE ANALYSIS.—The alfalfa samples, following thawing, were analyzed for residues by the electrometric method of Curry (1956), as adapted from the method of Hensel *et al.* (1954), based on the inhibition of cholinesterase activity of human blood plasma. This method was also used and described by Lilly *et al.* (1958). Individual samples of 150 gm. fresh weight were blended with a minimum amount of cold distilled water, from 150 to 350 ml. as needed, and strained through several layers of cheesecloth. Determinations and calculations on the liquid extract then followed the described method. For each treatment analysis, five 150-gm. samples were taken at random from the 2-pound composite sample from the treated plot and two 150-gm. samples were used from the check plot sample. Three 1-ml. aliquots from each 50-ml. incubation volume containing an aliquot of the extract from the 150-gm. sample were used for pH determinations.

Samples for dry-hay-residue analysis were obtained by randomly selecting plant material from a total of five places in two bales of treated hay and from three places in a single bale of untreated hay. These were bulked by treatment and ground in a Wiley mill. Five 100-gm. portions from the treated hay and two 100-gm. portions from the untreated hay were individually blended, each with 600 ml. cold distilled water, to produce the plant extracts required. Aliquots from each blending were handled in the same manner as were those from the fresh samples.

Technical phorate (99+%) was furnished by the American Cyanamid Company. Human blood plasma was extracted from outdated whole blood obtained from the local hospital and from a blood bank. In some instances frozen plasma was obtained and used.

In trials where known amounts of phorate were added to untreated alfalfa samples, the total amounts added could never be accounted for by the inhibition method. In fact, only about 50% of the phorate could be accounted for when it was added as a part of the water for blending. About 75% of the phorate could be recovered when addition was made to the extract after blending and straining through cheesecloth. Similar recovery difficulty has been reported for demeton in alfalfa (Annual Report of Cooperative Regional Project W-45, 1958). The reason for this loss is unknown, other than the possibility of substances in alfalfa apparently binding or reacting with this type of insecticide or its metabolites.

ANIMAL FEEDING TEST.—Fifteen Mexican-bred grade steers about 18 months old were used for the feeding test. Three pens of 5 steers each were selected from 25 animals so that each group of animals would be as similar one to another as possible. Weights of individual steers were taken on July 25 after a 4-day period to allow them to become adjusted to their new environment.

Feeding of hay from the experimental plots was started on August 3. The three pens of animals were fed the 1-pound treatment hay, the 4-pound treatment hay, and the untreated hay, respectively. The animals were fed as groups twice a day all the hay they would clean up, as well as a supplement of steamed rolled barley. The average amount of hay fed per animal per day was 15 pounds dry weight, while barley was fed initially at the rate of $1\frac{1}{2}$ pounds per animal per day. The rate for barley was gradually increased until each animal was getting 4 pounds per day by the third week of the trial. The two pens of steers which were fed treated hay, starting August 3, were kept on this diet for a month, after which they were fed hay from the untreated polt at the same rate as were the check pen of steers throughout the entire feeding period. This procedure was carried out to determine whether the animals fed the treated hay would be capable of gaining at the same rate when returned to untreated hay as would the check group.

Besides the initial weighing on July 25, before the treatment feeding period started, each steer was weighed on August 25, 22 days after the initiation of feeding of treated hay. Final weights were taken on each steer on September 24, again 22 days after feeding of treated hay had stopped.

A 10- c.c. blood sample was taken from the jugular vein

Table 1.—Residues recovered from alfalfa samples at various intervals following broadcast treatment with 2% granulated phorate and followed by flood irrigation.

Days After Application	Pounds oer Acre	
	1	4
1	374 ± 141[a]	244 ± 82
3	390 ± 236	520 ± 225
5	143 ± 11	380 ± 80
7	19 ± 3	113 ± 5
14	0	82 ± 12
21	0	98 ± 6
25	0	11 ± 3
Dry Hay[b]		0

[a] Each figure is p.p.m. on a fresh weight basis with the 95% confidence limit set by the standard error of the mean and the appropriate t value. Figures are derived from 5–150-gm. samples.

[b] Alfalfa was cut 27 days after treatment, sun dried, and baled. Samples for analysis were taken approximately 1½ months after baling.

of each animal preceding the treatment period and at intervals during the experiment. The first samples were taken on July 25 and subsequent samples at 1, 3, 5, 7, 14, 22, 30, 36, 43, 50, and 57 days after initiation of feeding of treated hay. The samples were held approximately 1 hour in a refrigerator and then placed in a freezer, where they were held for later cholinesterase determinations. All samples were taken at approximately the same time of day at each date.

Cholinesterase Determinations.—Blood samples taken from the cattle during and following the period when treated hay was fed were subjected to cholinesterase determinations by the bovine whole-blood method reported by Robbins *et al.* (1958). Duplicate determinations of enzymatic hydrolysis of acetylcholine were made on each blood sample, each corrected for nonenzymatic hydrolysis by an individual blank.

The blood samples were not treated with an anticoagulant at sampling time and thus were rather severely clotted when thawed following storage. Cholinesterase activity of the unclotted portions of the samples was extremely low, comparable to the rate reported for plasma alone (Robbins *et al.* 1958). It was found that the activity of the samples could be restored by homogenizing them with a Teflon-pestle tissue grinder to macerate the clot. Heparin, at the rate of 0.2 mg. per milliliter, was added to each sample just prior to homogenization to prevent further clotting. Cholinesterase activity was determined on portions of fresh blood samples that were purposely allowed to clot and then homogenized. Activity of these portions was essentially no different from that of portions from the same samples that had been initially heparinized. Furthermore, it was shown by comparing frozen and unfrozen portions of the same blood sample that the freezing period had no appreciable effect on cholinesterase activity.

Results and Discussion.—*Phorate Residues.*—The amounts of residues detected in the hay samples are shown in table 1. As would be expected, residues were extremely high in hay from both treatments shortly after application, the maximum being detected on the third day. At the lower treatment level residue concentration dropped off rapidly after the fifth day and no detectable residues were found after the 14th day. Residues remained rather high through the 21st day in material from the higher treatment rate and detectable amounts were found in the sample taken on the 25th day. The amount found in the last sample is somewhat questionable, however, since the inhibition percentage leading to its calculation was on the lower limit of the valid part of the standard curve. No detectable residues were found in the dry hay samples. These results are in general agreement with those of Lilly *et al.* (1958) for residues in barley, oats, and wheat after approximately the same applicable time intervals, although their plant material was initially planted in phorate-treated soil. Bowman & Casida (1957) were able to detect cholinesterase inhibiting material in vegetables 17 and 32 days after soil application of rates of less than 4 pounds per acre.

Since the standard curve for converting percentage inhibition to amount of phorate was prepared on the basis of actual phorate, the residue data are reported as such. It is known, however, that phorate is metabolized to various analogues in plants, the latter being more potent inhibitors of cholinesterase (Bowman & Casida 1957, 1958, Metcalf *et al.* 1957). In view of this fact, the data reported probably denote lower actual concentrations and lower but more inhibitory metabolite concentrations. No attempt was made to distinguish between phorate and its metabolites in the present work.

The standard errors of the means for each sampling date were generally large, as indicated in the confidence intervals reported in table 1. This variability indicates the difficulty encountered in obtaining uniform insecticide coverage in the application procedure, and further the difficulty in securing representative samples and subsamples for the residue analyses. The early samples in particular show a great deal of variability.

Feeding Experiment.—The weight data from the three groups of steers are shown in table 2. The results are inconclusive but nevertheless indicative. During the first weight-record period, the group of steers fed hay from the 4-pound treatment plot gained at a lesser rate than the group fed the 1-pound treated hay, and these in turn at a lesser rate than the check group. These differences in mean rates of gain were not significant. The mean differences were smaller during the latter weight period, but there were no striking changes in either of the treated groups. Rather, the mean rate of gain for the untreated group decreased. The reduction in number of individuals for this period makes a comparison almost meaningless. The small or negligible changes in the two treated groups upon cessation of the treatment indicate the absence of a recovery, providing there was possibly an initial effect during the treatment period.

A point of interest in reference to the weight gains is the observation that the animals fed on hay treated at the 4-pound rate found their hay somewhat distasteful. They ate it reluctantly and took considerably longer to clean up their ration than the steers in the other two groups. In addition, more hay was thrown out of the feed bunk by this group than by the others. There exists the possibility that these animals found this hay unsatisfactory because of an unfavorable taste or smell or both, owing perhaps to a trace of phorate or one or more of its metabolites. The latter have been shown to exist in plants treated with phorate (Bowman & Casida 1957, Metcalf *et al.* 1957). No

Table 2.—Rates of gain in weight of cattle fed hay made from untreated and phorate-treated alfalfa.[a] 1958.

Kind of Hay	Steer No.[b]	Gain per Day (Lb.) 7/25 to 8/25	8/25 to 9/24	7/25 to 9/24
Untreated	3	3.27	3.48	3.37
	4	3.80	2.93	3.37
	457	3.00	1.86	2.44
	459	3.33	−0.58[c]	1.41[c]
	461	2.70	—[d]	—
	Average	3.22	2.76	3.06
Phorate (1 lb./A.)	1	2.47	2.07	2.27
	93	2.97	2.59	2.78
	95	2.50	2.38	2.44
	96	1.33	—[d]	—
	467	3.60	3.31	3.46
	Average	2.57	2.59	2.74
Phorate (4 lb./A.)	94	2.67	2.86	2.76
	458	2.67	3.34	3.00
	460	1.30	—[d]	—
	464	1.60[e]	0.86	1.24
	465	2.30	2.17	2.24
	Average	2.11	2.31	2.31

[a] Hay from treated alfalfa was fed to the two groups from 8/3/58 to 9/2/58.
[b] Initial weight ranges of the cattle groups were: Untreated group, 442–551 lb.; 1-lb. group, 442–532 lb.; 4-lb. group, 475–545 lb.
[c] Steer went off feed 8/29/58 and remained sickly rest of period; included only in first month's averages.
[d] Slaughtered 9/2/58.
[e] On 8/20/58, steer showed illness symptoms of stiffness of hind legs, some frothing at mouth, obvious stomach distress, head held below shoulders, lackluster appearance of eyes, lassitude, poor reaction to external stimuli, and rectal temperature of 104° F. for 3 days. Treatment of ½ grain atropine was administered on 9/18/58. Apparent recovery was quite rapid.

means were used for determining actual consumption of hay, but this indication of unpalatability could help to explain the apparent reduction in rate of gain.

Table 4.—Analysis of variance of cholinesterase activity of blood samples from cattle fed hay made from untreated or from alfalfa treated with 1- and 4-pounds of phorate per acre.

Source of Variation	D.F.	S.S.	M.S.	F.
Total	209	18.7015		
Sampling dates	6	3.3867	0.5644	12.77**
Treatments	2	3.6986	1.8493	3.10
Check vs. treatments	(1)	(3.6867)	3.6867	6.18*
1 lb. vs. 4 lbs.	(1)	(0.0119)	0.0119	—
Animals/treatments	12	1.1105	0.0925	2.09*
Animals/treatments×date	72	3.1865	0.0442	29.47**
Duplicates	105	0.1587	0.0015	

* Significant at 5% level.
** Significant at 1% level.

Examination of the carcasses of slaughtered animals, one from each group at the end of the first weight period, revealed no gross pathological symptoms indicative of organophosphate toxicity. One particular steer in the group receiving 4-pound treated hay, number 464, is of interest because of the appearance of illness symptoms on August 20. These symptoms were tentatively diagnosed as possibly caused by phosphate poisoning, and atropine was injected for treatment on September 18. The individual subsequently recovered but the possibility of phosphate toxicity remains doubtful, as may be shown by the determinations of cholinesterase activity of the blood samples.

The data from this limited feeding trial do not show any significant effect from the feeding of alfalfa hay that had received phorate treatment. However, some evidence exists to doubt the complete lack of an effect from the feeding of 4-pound treated hay. Since the effective rate for aphid control is 1 pound per acre, rather than 4 pounds,

Table 3.—Cholinesterase activity[a] of blood samples from steers fed hay made from untreated and phorate-treated alfalfa.[b] 1958.

Kind of Hay	Steer No.	Dates Blood Samples Taken 7/25	8/4	8/6	8/8	8/10	8/18	8/25	9/1	9/8	9/15	9/22	9/29
Untreated	3	1.54	0.88	1.11	1.52	1.13	1.34	1.49	1.66	1.21	1.29	1.47	1.47
	4	1.92	1.83	1.46	1.83	1.41	1.55	1.82	2.02	2.05	1.57	1.86	1.71
	457	1.52	1.05	1.28	1.37	1.15	1.32	1.51	1.72	1.47	1.89	1.44	1.50
	459	1.92	2.08	1.45	1.99	1.68	1.79	2.13	2.14	1.92	1.93	1.79	1.76
	461	1.71	1.22	1.42	1.71	1.53	1.68	1.90	2.06	—[c]	—	—	—
	Ave.	1.72	1.41	1.34	1.68	1.38	1.54	1.77	1.92	1.66	1.67	1.64	1.59
Phorate (1 lb./A.)	1	1.50	1.29	1.28	1.33	1.36	1.37	1.49	1.66	1.65	1.11	1.55	1.53
	93	1.76	1.16	1.38	1.48	1.38	1.27	1.98	1.72	1.84	1.50	1.84	1.72
	95	1.27	1.15	1.13	1.49	1.05	1.22	1.35	1.41	1.19	1.48	1.07	1.06
	96	1.36	1.17	1.34	1.20	0.98	1.02	1.08	1.51	—[c]	—	—	—
	467	1.51	1.14	1.04	1.29	1.08	1.16	1.31	1.43	1.23	1.45	1.09	1.17
	Ave.	1.48	1.18	1.23	1.36	1.17	1.21	1.44	1.55	1.48	1.39	1.31	1.37
Phorate (4 lb./A.)	94	1.26	1.09	1.06	0.98	0.95	1.07	0.95	1.14	0.97	1.13	1.08	0.92
	458	1.84	1.41	1.43	1.65	1.24	1.51	1.93	1.66	1.68	1.63	1.56	1.53
	460	2.06	1.29	1.08	1.33	1.18	1.31	1.01	0.97	—[c]	—	—	—
	464	1.48	1.18	1.09	1.29	1.28	1.32	1.38	0.98	—[d]	1.11	1.48	1.06
	465	1.89	1.25	1.45	1.72	1.31	1.43	1.47	1.76	1.47	1.68	1.29	1.14
	Ave.	1.71	1.24	1.22	1.39	1.19	1.33	1.35	1.30	1.37	1.39	1.28	1.16

[a] Activity expressed as micromoles acetylcholine hydrolyzed by 0.05 ml. whole blood in 10 minutes. Each figure is an average of duplicate determinations; greatest range was 0.10 micromoles.
[b] Alfalfa cut 27 days after treatment, sun-dried, and baled. Fed to cattle 8/3/58 to 9/2/58.
[c] Steers numbered 461, 460, and 96 were slaughtered 9/2/58.
[d] Blood samples lost by breakage.

there is no evidence contrary to using phorate at this rate on established alfalfa stands.

CHOLINESTERASE DETERMINATION.—Table 3 shows the cholinesterase activity determined from blood samples taken at intervals from the three groups of steers fed hay from the two treated plots and the check plot. Duplicate determinations for individual samples agreed very well, the maximum discrepancy being about 7%. The general magnitude of these figures agrees very well with that of the figures reported by Robbins *et al.* (1958) for various types of cattle and with that of the figures reported by Petty & Lovell (1958), although the latter workers used erythrocytes in saline solution rather than whole blood. The cholinesterase levels reported here are greater than those reported by Hermenze & Goodwin (1959) when the two sets of data are compared on the same units of activity. However, comparison is difficult because of the difference in method of measurement, reaction temperature, substrate concentration, and blood fractions. The blood cholinesterase activity was quite variable between animals, as has been previously reported (Robbins *et al.* 1958, Petty & Lovell 1958, Hermenze & Goodwin 1959). It is also apparent from the data that blood samples from individual animals varied in cholinesterase activity from one sampling period to another. Hermenze & Goodwin (1959) reported no change in erythrocyte cholinesterase activity in samples taken on a hot day as compared with former levels of the same individuals but did not mention the number of animals sampled in this manner. The limited data presented here indicate that differences do exist from time to time but the reason in unknown. It is conceivable that various environmental and physiological conditions could influence the blood cholinesterase levels.

The feeding of hay from treated alfalfa seems to result in no consistent pattern of cholinesterase activity of the blood samples that would indicate an effect from the presence of residues. The variability may be such as to obscure a minor effect, if present. There seems to be a general trend toward decreased activity in blood from the group fed 4-pound treated hay, but the decrease is not sufficient to show a significant effect, as shown in the analysis of variance of the data, table 4. In view of the variability encountered in the determinations, more samples would be required over a sufficient interval of time to establish normal cholinesterase levels in order to prove a significant trend.

It is of interest to examine the level of the sample from steer number 464, reported above as possibly showing symptoms of phosphate toxicity during the feeding period. There was no indication of reduced cholinesterase activity during this period that would bear out this diagnosis.

The data in general substantiate the results from the feeding trial showing that cattle may be safely fed alfalfa hay from fields treated after the previous mowing at the effective rate of 1 pound of actual phorate per acre. The possibility cannot be completely discounted that a deleterious effect on cattle either in weight gains or in blood cholinesterase levels, or both may result from the feeding of hay from alfalfa treated with phorate at the rate of 4 pounds per acre, although within the limits of the present work no definite relationship could be shown.

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Boll Weevil Susceptibility to Toxaphene, Endrin, and Guthion in Five Alabama Localities[1]

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ABSTRACT

Laboratory experiments were conducted in 1956 and 1957 to determine the susceptibility of the boll weevil *Anthonomus grandis* Boh., to toxaphene endrin, and Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3,-benzotriazin-3-(4H)-ylmethyl) phosphorodithioate) in five Alabama localities. The technical insecticides were dissolved in acetone and applied topically to 2-day-old weevils reared from cotton squares. Mortalities were determined at the end of 72 hours. Approximately 25,000 weevils were used in the experiments. During 1957, LD-50 values varied among populations from different localities as follows: for toxaphene, from 12.5 to 61.8 μg. per gm. of boll weevil; for endrin, from 0.8 to 3.5 μg. per gm; for Guthion, from 0.9 to 2.3 μg. per gm. There was no evidence of acute resistance of the boll weevil from any of five localities to any insecticide tested. Mortality variations between times of year and between the two years were as great as among locality groups.

Insecticides have been used to control cotton pests for many years. The chlorinated hydrocarbon insecticides, toxaphene, endrin, BHC, aldrin, dieldrin, and heptachlor, have been effective against the boll weevil. In recent years, however, the boll weevil in some areas has developed resistance to various chlorinated hydrocarbon insecticides.

The first indication of boll weevil resistance to chlorinated hydrocarbon insecticides was reported in 1954 in Louisiana by Roussel & Clower (1955). Roussel *et al.* (1956) found that resistance had persisted in the boll weevil during 1956. By early June, 1956, it was determined by topical applications that about two-thirds of the cotton acreage in Louisiana was affected by resistant weevils (Roussel & Clower 1957). Walker *et al.* (1956) reported that chlorinated hydrocarbon insecticides failed to give satisfactory control of the boll weevil in several fields of cotton in the Brazos River Valley of Texas. According to Fye *et al.* (1957), 163 times as much endrin was required to kill 50% of the boll weevils from Darlington County, South Carolina, as was needed for Georgetown weevils and 133 times as much as needed for Florence County weevils. Hamner & Hutchins (1957) reported that the boll weevil in the Delta area of Mississippi was resistant to toxaphene, endrin, and dieldrin at all dosage levels. There was no evidence of resistance to organophosphorus insecticides in any of the studies reviewed.

Studies were conducted during 1956 and 1957 to determine the susceptibility of the boll weevil to toxaphene, endrin, and Guthion® (*O,O*-dimethyl *S*-(4-oxo-3H-1,2,3-benzotriazin-3-(4H)-ylmethyl) phosphorodithioate) in five Alabama localities. Results of these studies are herein reported.

METHODS AND PROCEDURES.—Boll weevils used in these studies were reared from squares collected from Courtland, Auburn, Lowndesboro, Frisco City, and Fairhope. The locality from which weevils were collected near Fairhope had no previous record of insecticidal application for boll weevil control. Chlorinated hydrocarbon insecticides had been used extensively at the other four locations during the past 7 years. Growers began reporting difficulty in controlling boll weevil in these areas in July, 1956. For comparison purposes, a Louisiana susceptible population was established on the Gulf Coast Substation at Fairhope. Infested squares were collected and stored in screen wire rearing cages in the laboratory until the adults emerged. When emergence began, the weevils were collected daily and given fresh cotton squares for food.

A stock solution of each insecticide was prepared by dissolving the technical material in acetone. Dilutions were made from each stock solution to give a satisfactory dosage range. When the adult weevils were 2 days old, they were separated and weighed in groups of 10 of approximately the same size. Immediately after weighing, the weevils were anesthetized with CO_2 and each was dosed with 1 microliter of insecticidal solution, which was applied dorsally to the posterior region of the prothorax. An equal amount of acetone was applied to each weevil in the check. The device used in the dosing process was described by Owens & Haynes (1958). Two to five concentrations of each insecticide were used in each test. Treatments were replicated 9 to 15 times, depending on the availability of weevils. Approximately 25,000 weevils were dosed in this study. Treated weevils were given fresh squares daily for food and held at 80 degrees F. for 72 hours at which time final mortality counts were made. Adjustments were made for natural mortalities occurring in checks by use of Abbott's (1925) formula. Analysis of variance was computed on results of data collected in 1957.

RESULTS AND DISCUSSION.—Mortality data from toxicological studies conducted during 1956 are presented graphically in figures 1, 2, and 3. These data revealed no evidence of acute resistance of the boll weevil to any insecticide tested. In general, the greatest variation in per cent mortality among localities occurred at the lower dosage levels and tended to decrease as the dosage level increased.

Results of studies with toxaphene are presented graphically in figure 1. The greatest variation between localities at the LD-25, 50, and 75 of toxaphene was 10.7-, 6.1-, and 2.4-fold, respectively. Weevils collected at Fairhope and Courtland appeared to be easier to kill than weevils from the other localities at the lower dosage levels. At the higher dosage levels, there appeared to be no appreciable differences in mortality of weevils from the five locations.

Results of studies conducted with endrin on square-reared weevils are presented in figure 2. Approximately seven times more endrin was required to kill 50% of the Frisco City weevils than was required for the most susceptible weevils from Fairhope.

[1] Taken from a thesis presented by the senior author in June, 1958, to the graduate faculty, Auburn University, in partial fulfillment of requirements for the M.S. degree in entomology. Accepted for publication November 4, 1959.

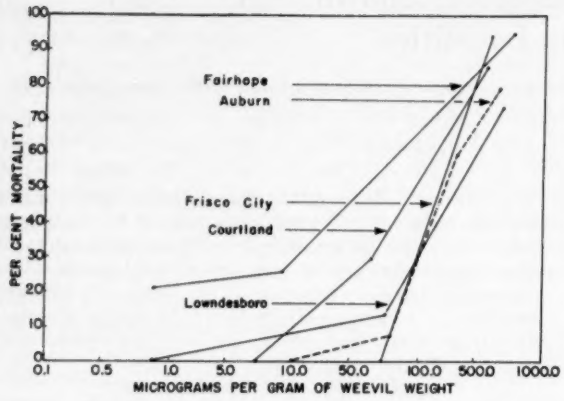

FIG. 1.—Effect of toxaphene on 2-day-old weevils from squares collected from different localities in Alabama, 1956.

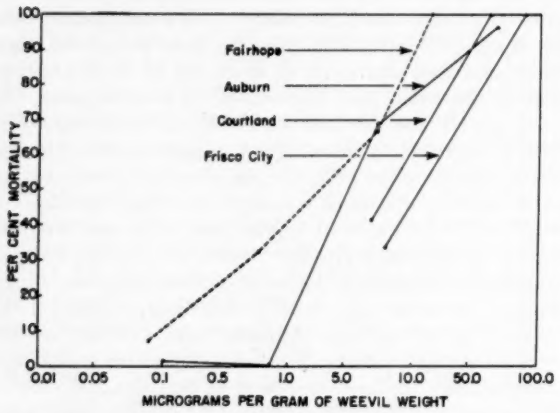

FIG. 2.—Effect of endrin on 2-day-old boll weevils reared from squares collected from different localities in Alabama, 1956.

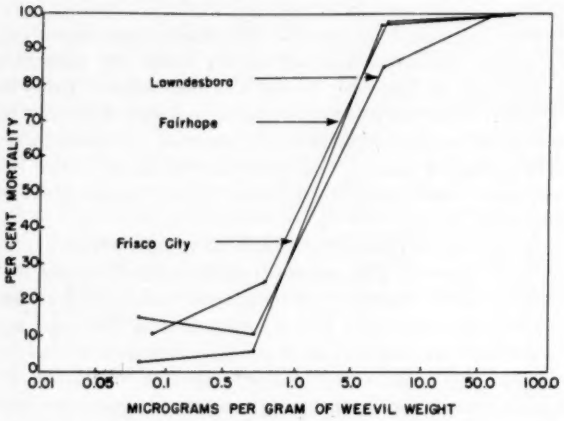

FIG. 3.—Effect of Guthion on 2-day-old boll weevils reared from squares collected from different localities in Alabama, 1956.

Mortality data on weevils from three localities treated with Guthion are presented in figure 3. Estimated LD-50's ranged from 1.8 to 2.9 micrograms per gram of weevil. These variations were considered negligible.

Data collected from toxicological studies during 1957 are presented in figures 4, 5, and 6. In experiments conducted in July using toxaphene (fig. 4) the Fairhope weevils, which appeared to be the most susceptible in 1956, were slightly harder to kill than weevils from the other localities. Weevils collected at Auburn appeared easiest to kill. Twice as much toxaphene was required to kill 50% of the Fairhope weevils as was required for those collected at Auburn.

The Courtland weevils were slightly more susceptible to endrin at all dosages than weevils from any of the other localities (fig. 5). Mortality variation tended to decrease as dosages were increased. Approximately three times as much endrin was required to kill 50% of the Fairhope weevils as was needed for the Courtland group.

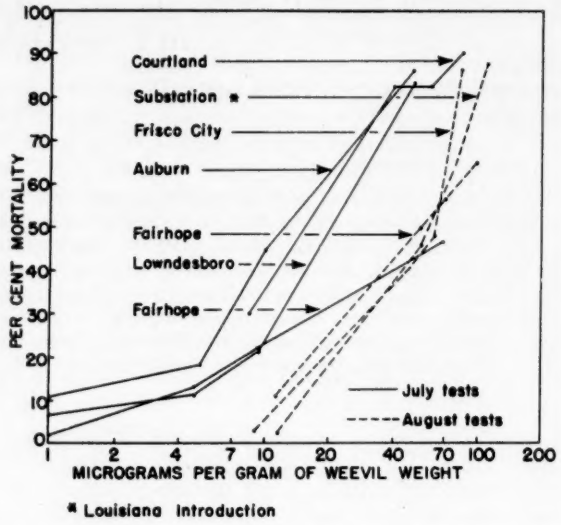

FIG. 4.—Effect of toxaphene on 2-day-old boll weevils reared from different localities in Alabama, July and August, 1957.

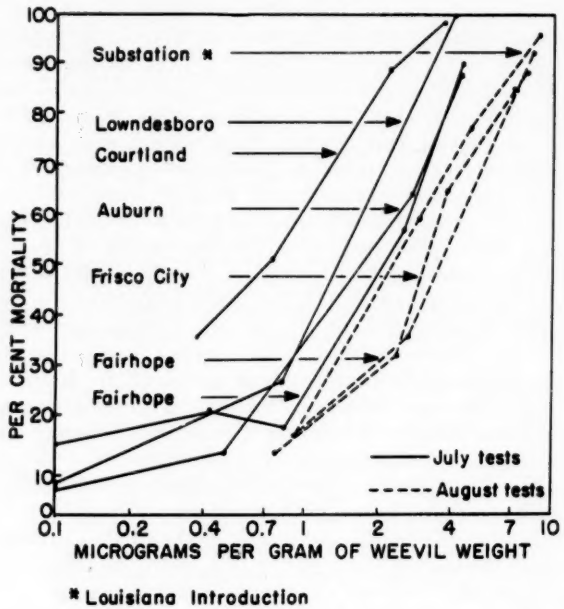

FIG. 5.—Effect of endrin on 2-day-old boll weevils reared from squares collected from different localities in Alabama, July and August, 1957.

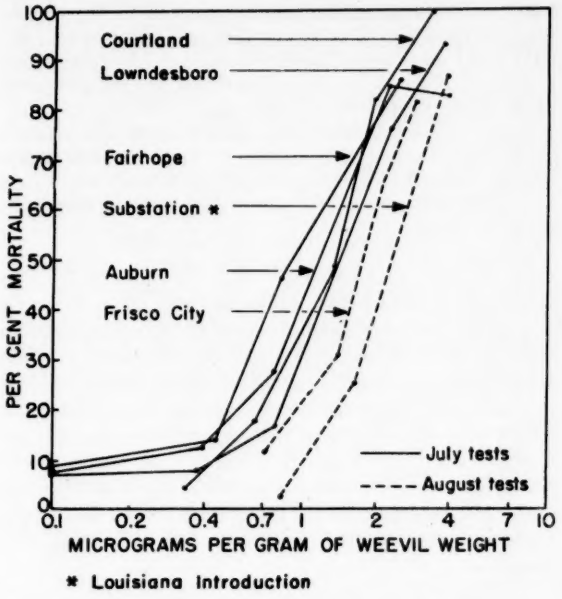

FIG. 6.—Effect of Guthion on 2-day-old boll weevils reared from squares collected from different localities in Alabama, July and August, 1957.

Results of tests with Guthion in July showed only slight variation in mortality among the weevils from the several localities (fig. 6). The amount of insecticide required to kill 50% of weevils ranged from 0.9 micrograms of insecticide per gram of weevil (Fairhope) to 1.5 (Lowndesboro).

Results of tests conducted during August using toxaphene are presented in figure 4. There were only minor differences among the three weevil groups at the 50% mortality level.

Results obtained in August with endrin indicated that only a slight variation occurred among the three weevil groups, especially at higher dosage levels (fig. 5). The Louisiana susceptible population established at the Gulf Coast Substation, Fairhope, in 1956 appeared to be slightly more susceptible to endrin in 1957 than the native weevils from Fairhope and Frisco City. The estimated LD-50's ranged from 2.3 micrograms of insecticide per gram of insect for the Louisiana weevils to 3.5 micrograms per gram for the native Fairhope weevils.

Only the Frisco City and Louisiana weevils were tested with Guthion during August (fig. 6). The Frisco City weevils appeared slightly more susceptible to Guthion than the Louisiana population at all dosage levels tested. The estimated LD-50's were 2.3 and 1.8 micrograms of insecticide per gram of weevil weight for the Louisiana strain and the Frisco City weevils, respectively.

Although the differences were not great, larger amounts of toxaphene and endrin were required to kill boll weevils in late August than in July (figs. 4 and 5).

Analysis of variance of data collected in 1957 revealed that Fairhope and Lowndesboro weevils were significantly more difficult to kill with toxaphene during July than Auburn weevils.

There were no outstanding differences between the mortality data obtained in 1956 and 1957, although considerable variation occurred. The greatest difference was only four-fold and could have resulted from different weather conditions and the use of a different generation of weevils. Variation between the 2 years and between times of year were as great as among locality groups. It is concluded that the 1956 and 1957 toxicological studies revealed no evidence of acute resistance of weevils collected from any locality to any of the insecticides studied.

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Field Studies of House Fly Resistance to Organophosphorus Insecticides[1]

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ABSTRACT

Applications of Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothiate) or ronnel (Korlan) for more than 5 years in New Jersey dairy barns resulted in decreased effectiveness in house fly (*Musca domestica* L.) control with these materials. Flies were collected from these barns and subsequently exposed in the laboratory on residual deposits of Diazinon, ronnel, DDT, and lindane. Resistance to these compounds was found in fly populations from these barns and from more than 20 other barns located up to 5 miles from the study barns. Resistance was less near the edge of the dairy farming area in the county and at the greatest distance from the study barns. Cross resistance to Diazinon, ronnel, lindane, and DDT

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was shown throughout the area, with highest resistance to DDT.

In the study barns a large population of the flies present before an insecticide application proved to have a lower level of resistance than the smaller number of flies present soon after an insecticide application. Thus, highest resistance was correlated with greatest amounts of insecticide in the barn and highest kills of susceptible flies. In one barn a similar reduction in fly population and an increase in resistance resulted when manure supporting heavy fly breeding was removed from box stalls. Observations in Sussex and Salem Counties also showed increasing Diazinon resistance and usually in proportion to Diazinon use. Some cross resistance to ronnel and high cross resistance to DDT and lindane were found in these areas. All evidence shows that DDT, lindane, and related materials will not give control where organophosphorus resistance exists.

Dimethoate (*O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate), and malathion were unsatisfactory in practical field control where resistance to Diazinon and ronnel was appreciable. At other locations this material gave 4 to 6 weeks of control.

Resistance of house fly (*Musca domestica* L.) to Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothiate) in New Jersey dairy barns was previously reported by Hansens (1958). Use of Diazinon in these same barns has continued so that changes in the resistance level could be studied. In some of the barns it was necessary to use other measures for fly control. Other barns were treated for a number of years with ronnel (Korlan) to study the development of resistance to that compound.

INSECTICIDE TESTS.—Several barns were selected for spraying with Diazinon or ronnel in 1959. The numbers used to designate barns and methods of treating and counting are the same as those used in previous papers (Hansens 1956, 1958).

Barns were sprayed using power equipment and a single-nozzle spray gun to wet surfaces to the point of run-off. Observations of results were made twice a week and a "fly index" determined. This index consists of the total flies counted on six stanchions (two sets of three in different parts of the barn) and six Scudder fly grids (18 inches square). When the index exceeded 60, control was considered unsatisfactory.

Barns 51 and 52 were treated for the first time in 1959. Results with residual sprays (table 1) showed that both Diazinon and ronnel were much less effective than reported previously. House fly resistance to Diazinon was first evident in 1957 and has increased in importance since then. Ronnel resistance of house flies in the field was shown in tests in 1958 and 1959. Dimethoate (*O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate) was used in the field in 1959 with variable results. Probably there is some cross-resistance to this insecticide in these locations were some organophosphorus resistance now exists. However, no flies were collected in the field and tested in the laboratory for resistance to dimethoate. Our tests indicate that this compound will not give satisfactory results where resistance to other organophosphorus compounds exists, but will give 4 to 6 weeks of control at other locations.

LABORATORY TESTS OF FIELD-COLLECTED FLIES.—*Monmouth County.*—For 2 years flies from several barns were collected at intervals and exposed to residual insecticides in the laboratory. Past history of insecticide treatments in these barns is known in detail. Barn 6 received two applications per year of 0.5% ronnel in 1955, 1956 and 1957; and two applications per year of 1.0% ronnel in 1958 and 1959. This barn is kept extremely clean and much of the fly problem is thus eliminated. Barn 8 received applications of ronnel as follows: 0.5% and 1.0% in 1956; three applications of 0.5% in 1957; two applications of 1.0% in 1958; and four applications of 1.0% in 1959. The history for barns 9 and 22 was given through 1957 in a previous paper (Hansens 1958). Barn 9 received one application of 1% Diazinon in 1958 and four applications in 1959. Barn 22 received one application of 1% Diazinon in 1958 and two applications in 1959; in addition this barn was treated once with malathion residual, for 6 weeks in 1958 with pyrethrum aerosols and in 1959 for 6 weeks with Dimetilan® (5-(3 methylpyrazolyl)N,N-dimethylcarbamate), bait on plastic strips and once with malathion residual. Barn 51 was constructed in the fall of 1958 and was treated four times in 1959 with 1.0% Diazinon.

As often as possible, but at irregular intervals, flies were collected in the barns and brought to the laboratory. From 15 to 40 flies were placed in cages and exposed to plywood panels (treated with known amounts of insecticide) for 15 or 60 minutes; they were then removed to untreated paper and fed, and 24-hour mortality was recorded. The cages were 5-inch embroidery hoops fitted with cellophane. The procedure was that described by Barber & Schmitt (1948). Insecticides were applied to panels at the following rates per sq. in.: Diazinon, 1.0 mg.; ronnel, 0.1 mg.; DDT, 1.0 mg.; and lindane, 0.1 mg. When sufficient flies were available, they were exposed by cages in duplicate tests on all four insecticides. Length of exposure depended on the insecticide used. These results (table 2) show a high degree of resistance with all four insecticides. The resistance of a fly population to Diazinon or ronnel in these barns resulted in cross resistance to other organophosphorus insecticides, as shown in these tests with ronnel and Diazinon (table 2) and by the failure of malathion to give control when it was used in Barn 22. The resistance level to chlorinated hydrocarbons, especially DDT, was much higher than to organophosphorus materials. Actually only a few tests were run with DDT because the mortality was little more than that in untreated lots of flies from the same barn. Thus, use of DDT, lindane, and other chlorinated hydrocarbons in these barns would be completely unsatisfactory because of the very high level of cross resistance to chlorinated hydrocarbons.

It will be noted that there was considerable variation in the level of resistance in any one barn on different dates. Many of these discrepancies can be explained if we examine these data for the fly population in each barn as measured by the fly index. The fly index for twice-weekly observations and the data from laboratory tests are shown graphically for barns studied in figures 1 through 4. The arrows in the figures indicate the dates of insecticide application (see table 1).

Barn 22 (fig. 1) was treated with 1% Diazinon twice without satisfactory fly control. Control was obtained for brief periods at four times in the season. One of these followed the second application of Diazinon but was of short

Table 1.—House fly control with insecticides in dairy barns in New Jersey. 1959.

Insecticide	Amount per 100 Gallons	Barn No.	Date Treated	Days Control
Diazinon 25%, w.p.	32 lb.	9	June 5	0
	32 lb.	9	July 10	14
	32 lb.	9	Aug. 14	14
	32 lb.	9	Sept. 1	13
	32 lb.	22	June 5	0
	32 lb.	22	June 30	6
	32 lb.	26	June 11	48
	32 lb.	26	Aug. 17	22
	32 lb.	51	Aug. 14	0
	32 lb.	51	Sept. 1	7
Diazinon 25%, emul. solution	2 gal.	48	June 18	5
	4 gal.	48	June 13	22
	4 gal.	48	Aug. 14	0
	4 gal.	25	June 11	29
	4 gal.	25	Aug. 17	14
	4 gal.	45a	June 11	28
	4 gal.	45a	Aug. 17	14
	4 gal.	45b	June 11	26
	4 gal.	45b	Aug. 17	14
	4 gal.	51	June 8	21
	4 gal.	51	July 13	0
Ronnel 25%, w.p.	32 lb.	6	June 5	45
	32 lb.	8	June 5	30
	32 lb.	8	July 13	18
	32 lb.	8	Aug. 14	0
	32 lb.	8	Sept. 1	8
Ronnel 24%, emul. solution	4 gal.	6	Aug. 14	14
	4 gal.	6	Sept. 1	8
Malathion, 59 SF	4 gal.	22	Aug. 28	0
	5 gal.	24	Aug. 18	0
Dimethoate	4 gal.	3	July 27	49
	4 gal.	4	July 27	18
	4 gal.	4	Aug. 18	28
	4 gal.	24	Sept. 1	14+
	4 gal.	52	July 13	29
	4 gal.	52	Aug. 28	7
Dimetilan strips	30 strips	22	July 27	0
	10 strips	5	July 27	0[a]
	20 strips	12	July 24	0[a]

[a] Many flies killed but only partial control. Space sprays were also necessary.

duration. There was an extremely rapid rise in the fly population at this time because of very heavy breeding in box stalls. These stalls were cleaned on July 8 and the fly population showed first a very rapid decrease and then started to build up again. Flies were collected from this smaller fly population and tested in the laboratory. These flies showed an increased level of resistance which probably was apparent only because of a changed ratio between resistant and susceptible individuals. The removal of breeding material resulted in a reduction of the total fly population containing both resistant and susceptible individuals. The susceptibles were then killed by the residual of Diazinon in the barn, with the result that tests of flies collected from the barn then showed a larger proportion of resistant individuals. Previously the breeding potential was so great that there was always a large proportion of susceptibles which had not yet received a lethal dose of insecticide. Also from laboratory observations it appears that resistant flies require as much as 24 to 48

Table 2.—Percentage mortality of field-collected house-flies in laboratory exposure[a] to insecticides at the treatment (mg./sq. in.) indicated.

Source and Date	Diazinon 1.0	Ronnel 0.1	DDT 1.0	Lindane 0.1	
				15	60
Laboratory strain	100	100	95	100	—
Field collections					
Barn 6					
July, 1958	69	43			
Oct.	71	17			
June 5, 1959	89	14	13	18	—
June 22	—	6			
July 27	18	7			
Aug. 5	44	5			
Aug. 31	36	30			
Sept. 10	71	—			
Barn 8					
July, 1958	85	33	0	13	—
Aug.	—	19	1	—	—
Oct.	85	5			
May 29, 1959	80	5			
June 5	70	24			
22	76	19	0	21	—
30	50	4			
July 8	79	5	0	12	62
27	25	0			
Aug. 5	38	8			
31	60	22	—	—	0
Sept. 9	96	14	—	—	47
Barn 9					
June, 1958	100	—			
Aug.	10	—			
Oct.	85	6	3	15	—
May 29, 1959	50	0			
June 5	57	80			
22	53	9	0	20	—
30	18	7			
July 8	55	6	0	15	—
23	23	—	—	—	17
27	7	—	—	—	—
29	58	3			
Aug. 5	26	0			
31	35	16			
Sept. 10	48	13			
Barn 22					
June, 1958	72	—	—	—	—
July	12	20	1	20	—
Aug.	6	—	—	—	—
Oct.	60	7	—	23	—
May 29, 1959	100	100			
June 5	99	16			
22	48	12	3	12	46
30	35	25	6	20	44
July 8	47	0	0	8	26
23	23	—	—	—	17
Aug. 5	26	6			
26	25	3	—	—	11
Sept. 4	43	9			
10	54	33			
Barn 51					
June 8, 1959	95	5			
11	91	—	4	13	13
22	81	24	4	22	42
July 8	72	2	0	20	33
27	15	5	—	—	3
Aug. 5	27	7	—	17	40
26	9	4	—	—	5
31	40	11	—	—	91
Sept. 10	15	7	—	—	26

[a] All exposures were for 15 minutes; a 60-minute exposure was also made with lindane (results given in last column).

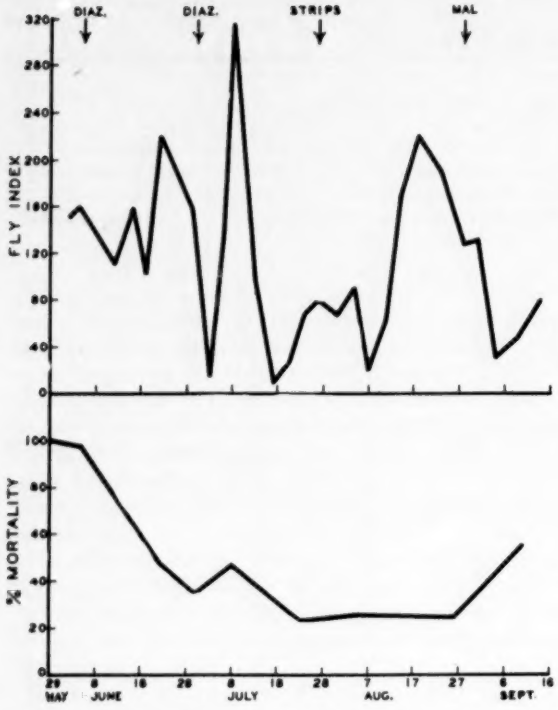

FIG. 1.—Comparison for Barn 22 of fly populations in the barn with mortality in the laboratory of field-collected flies.

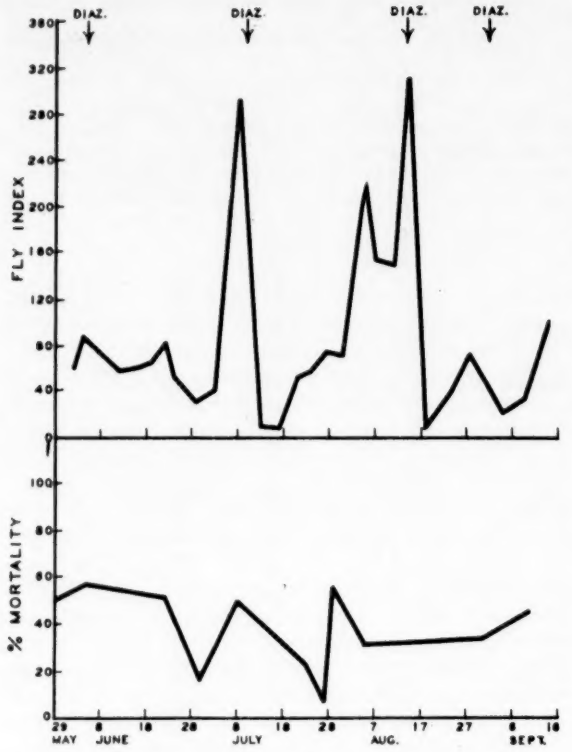

FIG. 2.—Comparison for Barn 9 of fly populations in the barn with mortality in the laboratory of field-collected flies.

hours longer to complete larval development and consequently fewer total flies are produced in the season. Further study needs to be made of larval development and behavioral differences in susceptible and resistant flies in the field.

Dimetilan-treated plastic strips were installed and while they helped by killing many flies, they did not give control. The application of a residual spray of malathion also did not give control. The decrease in fly population early in September resulted mostly from low temperature and not from the spray. The level of mortality in the lower graph (fig. 1) shows a decrease in mortality (increase in resistance) through the season with a slight rise in mortality with the large population early in July. The increase in mortality in early September probably was not actually caused by a loss of resistance but simply by longer life of susceptible flies because of the slower action of insecticides with cooler weather.

Similar conclusions may be drawn for Barn 9 (fig. 2) where 1% Diazinon was applied four times with a reduction in the fly population each time. Also, mortality of field-collected flies was highest in laboratory tests when fly populations were highest. At this location, fly control was obtained through more of the season because of low fly populations, even though the level of resistance was rather high. Four applications of Diazinon gave much poorer fly control than two applications per year from 1953 through 1957.

In the case of ronnel, in barn 8 (fig. 3) four applications of the insecticide were made and control was achieved through much of the season, especially the early part.

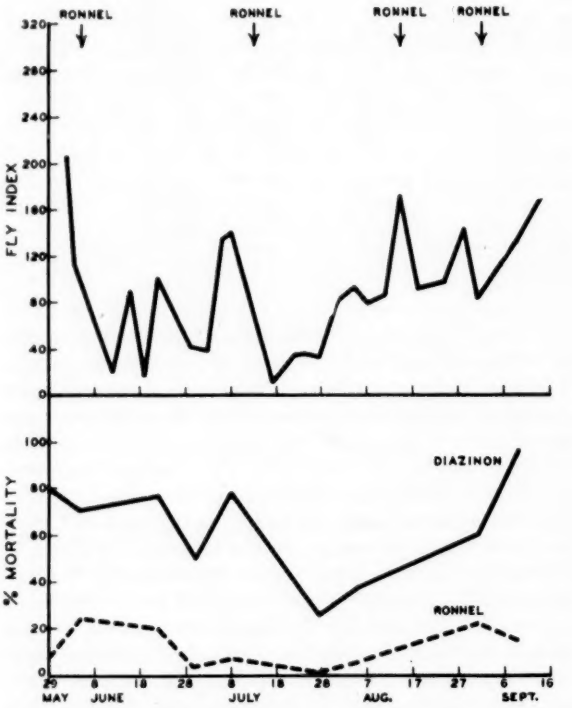

FIG. 3.—Comparison for Barn 8 of fly populations in the barn with mortality in the laboratory of field-collected flies.

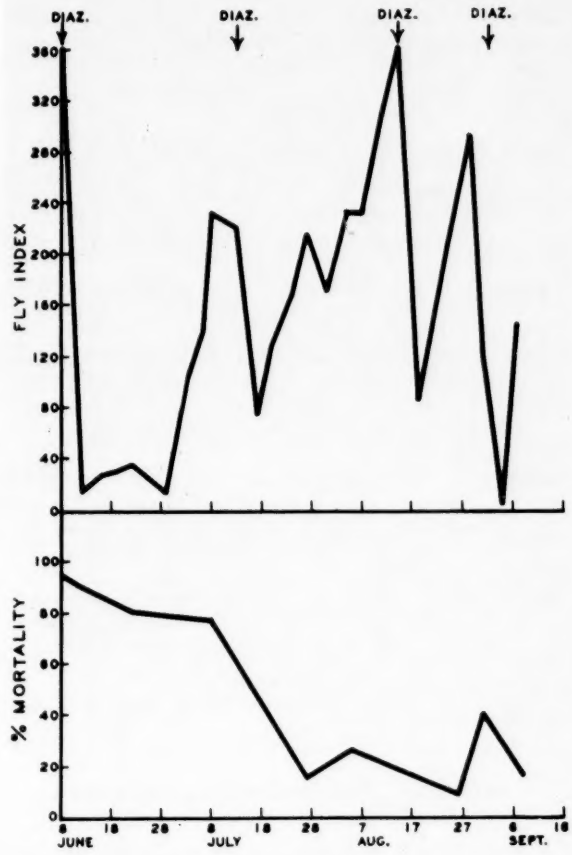

FIG. 4.—Comparison for Barn 51 of fly populations in the barn with mortality in the laboratory of field-collected flies.

Some resistance to ronnel was demonstrated in barn 8 and barn 6, but it was not sufficiently great to result in complete failure to control flies. More applications of ronnel were necessary than in previous seasons. Laboratory tests against Diazinon and ronnel showed again a parallel between mortality and fly populations, with higher mortality when larger populations exist in the barn.

Barn 51 (fig. 4) was newly built and first sprayed in 1959. Even with four applications of Diazinon, satisfactory fly control was not obtained. At this location there was extremely heavy fly breeding in the barn and even in early June there was an extremely large adult fly population. The first insecticide application resulted in excellent control for 2 weeks. Subsequent sprays gave poorer results. The mortality for flies tested in the laboratory showed a decrease as the season progressed, and before the end of the season low kills and considerable resistance to Diazinon. It should be noted that the laboratory test in early June showed a slight amount of resistance in the population.

In addition to these studies flies were collected in more than 20 barns in Monmouth County at distances up to 5 miles from the study barns. Flies were tested in the same fashion on insecticide-treated panels and mortality noted. Insecticides used were Diazinon, ronnel, DDT, lindane and malathion. Collections were not numerous enough at these farms to permit detailed analysis of the data, but certain trends were evident. There was some degree of resistance to organophosphorus insecticides at all farms surveyed and a higher degree of resistance to chlorinated hydrocarbons, the highest to DDT. Geographically the lowest resistance was found farthest from the barns which were sprayed experimentally, *i.e.*, barns 6, 8, 9 and 22. These more distant locations are also at the edge of the dairy farming area in the County.

Other Areas.—Two other areas in New Jersey have been surveyed for house fly resistance in relation to use of Diazinon in dairy barns. At the Dairy Research Farm at Beemerville, Diazinon has been used since 1955 with 1 or 2 applications per year. Excellent control was obtained until 1959 when some failure to control was noted. Tests in the laboratory of flies collected at Beemerville each month in 1959 showed that a larger and larger portion of the population was resistant to Diazinon and the other materials tested.

In Salem County, Diazinon was used as a residual spray in two barns for four seasons, 1954 through 1957, but not during 1958 and 1959. Another barn was treated with organophosphorus fly baits during the same four seasons. Collections of flies in 1959 showed some degree of resistance to Diazinon, ronnel, DDT, and lindane, but this resistance was appreciably less than in Monmouth County where Diazinon has been used more extensively. The highest resistance was in the fly population in barns where the greatest number of Diazinon residual sprays had been applied.

In New Jersey further studies are planned on the interrelation of fly populations and resistance, including cross-resistance, to a variety of materials. At the present time the outlook for fly control without recourse to sanitation and mechanical measures is not very bright. Chlorinated hydrocarbons are of virtually no value. Organophosphorus materials are decreasing in usefulness and even space sprays are less effective than previously. Of all the materials available, Diazinon is still giving best results in many locations and kills many flies even where resistance is present. It remains a question as to whether or not it will fail completely. Emphasis on sanitation becomes more important than ever in fly control as resistance problems increase in complexity. More emphasis needs to be placed on this aspect of fly control, both in research and in practical control.

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SCIENTIFIC NOTES

Discovery in Michigan of *Orgilus obscurator* (Nees), a Foreign Parasite of the European Pine Shoot Moth[1]

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Orgilus obscurator (Nees) (Hymenoptera, Braconidae) is a cosmopolitan parasite of the European pine shoot moth (*Rhyacionia buoliana* (Schiff.)) in Europe. In efforts to promote the natural suppression of the shoot moth in North America, this parasite has been imported from Europe and released at various times since 1928 in Ontario (Coppel & Arthur 1953) and in the northeastern United States (Clausen 1956). Kingsville, Ontario, about 25 miles from the Michigan-Ontario boundary, is the closest of any release points to Michigan.

Previous findings of *Orgilus obscurator* parasitizing the European pine shoot moth at some distance from release points (Coppel & Arthur 1953, Juillet 1957) prompted a search for this parasite in Michigan. Overwintering shoot moth larvae from 9 localities in Lower Michigan were carefully examined, and the parasite was found in the larvae from three localities, all in southeastern Michigan (table 1). Dr. A. P. Arthur, Entomology Research Laboratory, Belleville, Ontario, kindly verified the identity of the Michigan specimens.

Orgilus obscurator attacks very young shoot moth larvae and spends the winter as a tiny larva inside the body of its host.

Table 1.—Parasite examination records of third and fourth instar European pine shoot moth larvae in Lower Michigan.[1]

Locality	Species of Host Pine[2]	Number Shoot Moth Larvae Dissected	Number with *Orgilus obscurator*
Near Pinckney, Livingston County	Red	17	4
Near West Olive, Ottawa County	Red	100	0
Maybee, Monroe County	Red and Scotch	66	0
Near Lapeer, Lapeer County	Red	14	0
Pearl Beach, St. Clair County	Red	45	2
Near Boon, Wexford County	Red	100	0
Near Holland, Ottawa County	Scotch	67	0
Stinchfield Woods, Washtenaw County	Ponderosa	12	0
Port Huron, St. Clair County	Mugo	14	12

[1] Larvae were collected in 1957 and 1958 between August 4 and December 1.
[2] Red pine = *Pinus resinosa* Ait.; Scotch pine = *P. sylvestris* L.; ponderosa pine = *P. ponderosa* Laws.; mugo pine = *P. mugo* Turra.

It is easy to identify during the winter because at that time it has a very distinct form (illustrated by Arthur 1954). Moreover, none of the commoner native parasites which attack the shoot moth are present internally during the winter.

The fact that *Orgilus obscurator* was found in the eastern part of Lower Michigan suggests that it spread into Michigan from Ontario. However, before importations from Europe started it was recorded from the shoot moth at one locality in New England (Clausen 1956) and two localities in Ontario (Watson & Arthur 1959), which facts indicate that it may be introduced into areas at the same time as the host.

The value of this parasite in suppressing the shoot moth in the Michigan localities where it has been found has not been determined yet. However, its presence is an indication that it is adapted to the climate and the biological conditions of Michigan, and suggests that studies of this and other European parasites as suppressing agents of the shoot moth in the Lake States region should be intensified.

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[2] Stationed at the East Lansing Michigan Research Center. In cooperation with Michigan State University.

Artificial Medium for Rearing Some Lepidopterous Corn Insects[1]

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The rearing of phytophagous insects on media, rather than their host plant, is advantageous in a variety of investigations. Laboratory-reared larvae can be used for the study of insect pathogens, plant resistance factors, effects of insecticides, and study of radiation on fertility and growth—as well as for the study of life cycles of some little-understood insects.

During the course of investigations on corn insect pathogens and studies on insect life histories, it has been discovered that a medium ideal for laboratory rearing of the European corn borer, *Pyrausta nubilalis* (Hbn.), is satisfactory for the rearing of two additional corn pests—the corn earworm, *Heliothis zea* (Boddie), and the black cutworm, *Agrotis ipsilon* (Hufn.).

The medium used was that of Bottger (1942) and Beck *et al.* (1949) modified for our purposes. At 85° F. corn borers pupate on this medium within 14 to 17 days after fertile eggs in the black-head stage are placed on it. The components, in milligrams per gram, of this medium are as follows:

Water	833.10
Agar (Difco-Bacto Agar)	22.16
Cellulose (powdered)	11.08
Glucose	34.70
Casein (vitamin free)	34.70
Cholesterol	2.72
Corn oil plus 1% α tocopherol	1.36
Salts mixture #2, U.S.P. XIII	4.18
Choline chloride	0.39
Brewers yeast	27.82
Ground alfalfa	27.82
Total	1000.03

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When this medium is used for the rearing of corn earworms, larvae require 15.5 days to grow from 2nd instar to pupation when held at 85° F. Black cutworms require 31 days from egg to pupation. Newly hatched corn earworm larvae do not survive on this medium, but when fed corn silks until after the first molt, growth is nearly normal.

The use of this medium presents the possibility of rearing other plant-feeding insects in the laboratory for many purposes. Newly hatched armyworm, *Pseudaletia unipuncta* (Haw.), larvae were able to feed sufficiently to pass through several instars, but no pupation occurred. Field-collected late instar fall armyworms, *Laphygma frugiperda* (J. E. Smith), completed their growth, pupated, and emerged from vials containing the above media. Field-collected alfalfa caterpillar., *Colias philodice eurytheme* Bdv., and white-veined dagger, *Simyra henrici* (Grote) apparently did not feed on the medium, but starved in the vials. Modifications in the physical consistency of the medium or addition of attractants may make it possible to rear a number of different species on essentially the same medium.

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Control of Army Cutworms in Alfalfa During 1959[1]

R. E. PFADT, *University of Wyoming, Laramie*[2]

Investigations by Burkhardt (1954) and DePew (1959) in Kansas and by Pfadt (1954, 1955) in Wyoming have revealed that several of the chlorinated hydrocarbon insecticides applied as emulsive sprays are effective agents in controlling infestations of the army cutworm, *Chorizagrotis auxiliaris* (Grote). These studies showed that five insecticides—endrin, dieldrin, DDT, TDE, and Dilan (1 part 1,1-bis(*p*-chlorophenyl)-2-nitropropane and 2 parts 1,1-bis(*p*-chlorophenyl)-2-nitrobutane) were particularly good. The results agree with laboratory tests by McDonald & Jacobson (1958) who demonstrated the high toxicity of endrin and dieldrin to the army cutworm. Although present recommendations appear adequate, the availability of newer chemicals and formulations prompted further research on the control of this cutworm in Wyoming.

The following insecticides were tested as emulsive sprays: endrin at 0.1 and 0.2 lb., dieldrin at 0.25 and 0.5 lb., Shell SD 4402 (1,3,4,5,6,7,8,8-Octachloro-3a, 4,7,7a-tetrahydro-4,7-methanophthalan) at 0.08 and 0.16 lb., Sevin® (N-methyl-1-naphthyl carbamate) at 1 and 2 lb., and Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*, 6,9,9*a*-hexahydro-6,9-methano 2,4,3-benzodioxathiepin-3-oxide) at 0.5 and 1 lb. per acre; the following insecticides were tested as granules: endrin at 0.2 lb. and dieldrin at 0.5 lb. per acre.

As cutworm infestations in Wyoming were light in 1959, considerable time was spent in finding a field with sufficient cutworms for testing. Plots were finally located in an alfalfa field having a density of 1.8 army cutworms per square foot on April 23, the day the treatments were applied. Although the field contained other kinds of cutworms, namely *Feltia ducens* (Wlk.), at a density of one third per square foot and two unknown noctuids in smaller numbers, only the army cutworm was considered in appraising effectiveness of treatments. Based on head capsule width, army cutworms were judged to be mainly in the sixth stadium at time of treatment and in the seventh 14 days later.

Table 1.—Effectiveness of five insecticides 2 weeks after application by ground sprayer for control of army cutworm in alfalfa, Huntley, Wyoming, May 7, 1959.

Spray Treatments	Actual Toxicant (Lb./A.)	No. Cutworms per 18 Sq. Ft.	Reduction (%)	No. Cutworms per 48 Sq. Ft.	Reduction (%)
Shell SD 4402	0.16	0	100	0	100
Thiodan	1.00	0	100		
Thiodan	0.50	0	100	3	94
Endrin	0.20	1	95	2	96
Shell SD 4402	0.08	1	95	7	86
Dieldrin	0.50	2	90		
Endrin	0.10	4	80		
Dieldrin	0.25	10	50		
Sevin	2.00	12	40		
Untreated		20	0	51	0
L.S.D. at 5% level		3.2		10.8	
at 1% level		4.4		15.4	

The infested field was divided into three blocks of thirteen 22×90 ft. plots each. Twelve treatments and one check were assigned at random to each block.

A tractor-driven sprayer fitted with a 20-foot boom and fan-type nozzles (TeeJet No. 65015) applied the emulsive sprays in a complete cover to the soil and vegetation at a rate of 12.25 gallons per acre and a pressure of 50 lb. p.s.i. A Gandy Hi-Lo Granular Chemical Applicator dispensed 2% endrin and 5% dieldrin granules (RVM type, 24 to 48 mesh Attaclay) in a single 12-foot swath per plot and at a rate of 9.2 lb. per acre.

Treatments were made in the afternoon between 12:30 and 4:00, the wind ranged between 0 and 5 m.p.h., and the temperature between 58° and 62° F. The soil was dry to a depth of ½ to ¾ inch but moist below this depth. Height of alfalfa ranged between ¾ to 3 inches. During the 15 days after treatment the minimum temperatures ranged between 30° and 52° and averaged 41° F., while the maximum ranged between 46° and 88° and averaged 67° F. Rain on the 11th, 12th, and 14th days amounted to 0.85, 0.34, and 0.57 inch, respectively.

Density of cutworms was determined by examining six 1-square-foot samples in each plot. The plots treated with SD 4402, Thiodan at 0.5 lb. per acre, and endrin at 0.2 lb. per acre, and the untreated plots were examined in greater detail by taking 10 additional 1-square-foot samples from each. Sampling was carried out on May 6 to 9, an average of 14 days after treatment, and results were evaluated by comparing the number of live cutworms found in treated and in untreated plots.

RESULTS.—The results, summarized in table 1, show that SD 4402, endrin, and Thiodan at all concentrations tested and dieldrin at the higher concentration provided effective control of the army cutworm. The differences in effectiveness among these treatments were not significant. Laboratory studies by Shell Development Company (1959) have demonstrated that SD 4402 has a greater toxicity than endrin to the southern armyworm, *Prodenia eridania* (Cram.), and to the corn earworm, *Heliothis zea* (Boddie). Although in our tests SD 4402 at 0.16 lb. gave 100% control while endrin at 0.20 lb. gave 96% control, further and more refined testing is necessary to show that SD 4402 is significantly better than endrin for field control of the army cutworm.

Granulated formulations of endrin at 0.2 lb. per acre and of dieldrin at 0.5 lb. per acre and a spray of Sevin at 1 lb. per acre did not provide any control of the army cutworm. Sprays of Sevin at 2 lb per acre and of dieldrin at 0.25 lb. per acre gave only poor control. Because the identity of the two concentrations of Sevin became lost in applying the treatments, it was assumed

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[2] The writer wishes to acknowledge with thanks the assistance of Clarence Tietmeyer in doing the experimental work and the help of H. W. Capps in identifying the species of cutworms.

that the 40% control was effected by the higher concentration (2 lb. per acre) of Sevin and that no control resulted by the application of the lower concentration (1 lb. per acre) of Sevin.

The response of army cutworms to Thiodan is perplexing, since DePew (1959) found this insecticide ineffective in controlling an infestation in sugar beets, but Pruess (1959) found it highly effective in controlling infestations in vetch and small grains, and the present writer found it effective in alfalfa.

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Small Amounts of Insecticides Effective in Controlling Grubs of the Japanese Beetle[1]

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Certain insecticides in very small amounts are effective in controlling grubs of the Japanese beetle. This is clearly indicated when the residues of chlordane, BHC, heptachlor, and aldrin in the soil (Lichtenstein & Polivka 1959) are studied in relation to the grub populations in the same series of plots (with the exception of those containing aldrin) (Polivka 1959, tables 2 and 7). The grub populations in the aldrin-treated plots do not appear in print.

The data in table 2 (Polivka 1959) show that 12 years after treatment with chlordane the grub population in the plots treated with 1 pound per acre was not significantly different from that in the untreated plots. This rate of chlordane ceased to be economically effective at the end of the sixth year but the grub population remained significantly different from that in the untreated areas throughout the period of the investigation except for the last sampling date (12th year). These data also indicate that the threshold of effectiveness for chlordane in the soil lies somewhere between 0.21 and 0.44 pounds per acre. This is the conclusion after finding that no significant control was obtained where only 0.21 pound per acre of the insecticide remained in the soil whereas 100% control occurred in all plots that had 0.44 pound or more remaining.

Grub populations in the BHC-treated plots in the 10-pound level only were significantly different from that in the untreated plots 12 years after application. While the population in the 10-pound rate of treatment has been significantly different from that in the untreated plot throughout the period of the study, a few grubs were found in these plots during the sixth and seventh years. The data also show that effective control was never obtained with the 0.25-pound rate, and that the 2.5- and 5.0-pound rates were not effective after the fifth year.

When soil from heptachlor-treated plots was analyzed 9 years after application, no heptachlor was recovered by chemical assay but it was found that heptachlor epoxide was present at the rate of 0.06 to 1.62 pounds per acre. According to the bioassay, these samples contained heptachlor at the rate of 0.04 to 1.47 pounds per acre when measured against a heptachlor standard. Since 100% control of the Japanese beetle grubs was still obtained in all treatments 9 years after the application, the population data indicate that very low soil residue levels of this material, either as heptachlor or as heptachlor epoxide, did control the grubs.

No aldrin was recovered by chemical assay from plots that were treated 4 years previously at the rate of 3 pounds per acre and only 0.14 pound per acre was indicated by bioassay when measured against an aldrin standard. When these samples were measured against a dieldrin standard, it was found that the 3-pound rate still had 0.31 pound as dieldrin per acre by chemical assay and 0.25 pound as dieldrin by bioassay. Despite this low rate of toxicant present in the soil, 100% control of Japanese beetle grubs was obtained throughout the 4-year period.

The grub populations and soil residues reported by Lichtenstein & Polivka (1959) and by Polivka (1959) indicate that chlordane, heptachlor, and aldrin, in minute quantities, are each highly effective in controlling grubs of the Japanese beetle. The tests with BHC, on the other hand, indicate that this material should not be recommended for control of Japanese beetle grubs.

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[1] Accepted for publication November 20, 1959.

Effect of DDT Resistance on the Development of Malathion Resistance in House Flies[1]

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The selection of house flies (*Musca domestica* L.) by exposure to malathion alone can result in the rapid development of a strong resistance to DDT, even in a colony of flies never exposed to the latter insecticide (LaBrecque *et al.* 1959), but selection with DDT seldom gives more than slight resistance to organophosphorus insecticides. To determine whether this slight resistance has any effect on the further development of resistance to organophosphorus compounds when they replace DDT in field usage, tests were conducted with two strains of flies, one susceptible to all insecticides and one highly resistant to DDT.

Two new experimental colonies were established, designated as the No. 1-M and the R-M colonies. The No. 1-M colony was started with flies from the Orlando No. 1 colony, which had been maintained in the laboratory since 1946, and subjected to 273 generations of selection with DDT. The flies of this colony were practically immune to DDT, and showed 3- to 8-fold resistance to malathion. The No. 1 colony was originally derived from the Orlando regular colony. The R-M colony was started with flies from the Orlando regular colony, which had been maintained in the laboratory since 1943; the regular colony had been started with flies collected in a local dairy before the first use of DDT, had never been subjected to insecticide pressure, and was susceptible to all insecticides.

Selection in both experimental colonies was accomplished in each generation by exposing 100 to 200 4- to 5-day-old flies of undetermined sex in a wind tunnel to 0.25 ml. of an odorless kerosene spray containing a concentration of malathion adequate to cause 90% to 95% mortality. After treatment the flies

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Table 1.—Resistance to malathion developed in regular (R-M) and DDT-resistant (No. 1 and No. 1-M) strains of house flies by selection with malathion (R-M), DDT (No. 1), or DDT followed by malathion (No. 1-M).

Generation	Ratio to Flies of Regular Colony					
	LC-50			LC-90		
	R-M	No. 1	No. 1-M	R-M	No. 1	No. 1-M
Parent	1.00	3.10	3.10	1.00	3.50	3.50
2nd	1.27	—	4.73	1.32	—	5.09
4th	<1.00	6.36	5.09	1.09	6.41	3.24
5th	1.12	1.82	6.37	1.23	2.85	6.97
7th	1.24	4.33	9.93	<1.00	4.10	9.14
9th	1.27	2.22	4.44	1.75	3.18	4.99
12th	1.60	2.66	11.24	1.88	3.25	12.91
14th	1.31	7.91	21.15	1.27	6.82	26.60
16th	1.64	5.56	40.87	2.38	5.06	25.51
17th	2.18	7.79	25.84	2.13	6.27	28.81
17th[a]	1.81	4.80	20.00	1.07	4.34	33.02

[a] Two series of tests in the 17th generation.

were held for 24 hours and the mortality was recorded. The survivors were then released in a rearing cage where oviposition medium was available. Twenty-four hours later the eggs were collected and placed in larval medium for rearing of the subsequent generations.

In the No. 1-M strain, the initial selective concentration was 4%. It was increased to 8% at the second generation, and then to 10% from the 8th to the 17th, whereas the concentration for the R-M remained at 2% for the first seven generations and was raised to 2.5% for the remainder of the experiment. It should be emphasized that, although the concentration of malathion used in the two colonies was not identical, the selection pressure in terms of the percentage of flies surviving the exposures was about the same. If identical concentrations had been used, the pressure would have been much lower in the No. 1-M colony than in the R-M colony.

As resistance developed, its progress was followed by exposing 4- to 5-day-old females in the wind tunnel to sprays containing a range of concentrations of malathion. After treatment the flies were transferred to screen holding cages and supplied with a 10% sugar solution on absorbent cotton pads. Mortality was recorded after 24 hours. Duplicate tests with 20 females were run at each concentration of insecticide. The LC-50's and LC-90's were computed from the concentration-mortality data, and the degree of resistance was determined from the ratio of these lethal concentrations to those derived from concurrent tests with flies of the parent colonies. The results are presented in table 1.

Resistance to malathion developed much more rapidly in the strain previously selected with DDT than in the regular strain. The No. 1 colony showed 2- to 8-fold resistance to malathion, and resistance in the No. 1-M colony increased to 20- to 40-fold by the 14th to 17th generations, whereas it remained below 3-fold in the R-M colony. All these colonies originally stemmed from the Orlando regular colony. In the No. 1 strain the selection with DDT had been in effect for many generations and at a very high level, but the results suggest that the widespread use of DDT predisposed field populations to the development of organophosphorus resistance.

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Thiodan, a Promising Chemical for Control of the Lesser Peach Tree Borer[1]

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Madsen (1959) reported data showing that Thiodan,® identified chemically as 6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide, was effective when used in two applications for the control of the peach tree borer, *Sanninoidea exitiosa* (Say), on apricots. Cochran *et al.* (1959) reported excellent control of the peach tree borer in peaches when using a wettable powder of Thiodan containing 25% of the active ingredient at a rate of 3.0 pounds per 100 gallons of water in a three-treatment schedule. Summers (1959) found that Thiodan gave promising results for control of the peach twig borer, *Anarsia lineatella* Zell., on almonds and peaches.

It is the purpose of this paper to summarize the results of a 1-year study in which Thiodan was tried for the control of the lesser peach tree borer, *Synanthedon pictipes* (G. & R.), on peach trees. This insect has a life history somewhat comparable to the peach tree borer (Smith 1951, 1952).

Methods and Materials.—The experiment was conducted on the Niagara Research Farm at Middleport during the 1958–59 season. Trees in the test orchard were 13 years old and were comprised about equally of the varieties Hale Haven and July Burbank. With one exception, namely, the "4-treatment" schedule, all plots were made up of a minimum of 10 trees and were replicated. In the case of the 4-treatment schedule, there were five trees per plot.

Sprays were applied with a conventional high pressure sprayer at about 600 p.s.i. Eight gallons of dilute spray were applied to each tree and special effort was made to thoroughly wet all bark surfaces. A single dosage of 3 pounds of a wettable powder of Thiodan containing 25% of the active ingredient per 100 gallons was used throughout the experiment. Three pounds of a wettable powder of parathion containing 15% of the active ingredient per 100 gallons were employed as the standard treatment. Dates of application for the various schedules are given in table 1.

Table 1.—Results of field tests for control of the lesser peach tree borer, *Synanthedon pictipes*. Gasport, New York. 1958–59.[a]

Material[b]	Date of Application	No. of Trees Sampled	Total No. of Borer Larvae	Per Cent Control Over Check
Thiodan, 25%	6/18	12	27	72
	6/18, 7/7	12	13	87
	6/18, 7/7, 7/28	12	7	93
	6/18, 7/7, 7/28, 8/18	5	3	93
Parathion, 15%	6/18	12	24	76
	6/18, 7/7	12	11	88
	6/18, 7/7, 7/28	10	3	96
	6/18, 7/7, 7/28, 8/18	5	0	100
Untreated	—	12	97	

[a] Data taken April 1959.
[b] Applied at the rate of 3 pounds of wettable powder per 100 gallons.

Infestation records were taken in April 1959. All injured areas on the trunk or scaffold limbs that could be conveniently reached from the ground were examined closely for borer larvae. Counts were made on about half the trees represented in all the plots.

Discussion of Results.—A general summary of the results is given in table 1. The single application of Thiodan or parathion

[1] Accepted for publication November 13, 1959.
[2] The author wishes to express his appreciation to Dr. E. H. Smith, Geneva, N. Y. for his advice in conducting and evaluating this experiment.

provided about 70% control, but this would not be considered commercially satisfactory. Appreciable improvement in control was noted in the two-treatment schedule. Three applications of Thiodan gave 93% control. There was some question as to whether a fourth application would afford sufficient added control to justify the treatment. More extensive testing will be required to resolve this point.

CONCLUSION.—Results from 1 year of field testing show Thiodan to be a promising chemical for the control of the lesser peach tree borer. On the basis of these results a schedule of three treatments per season would be required for commercial control.

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Toxaphene Residues in Hogs[1]

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Limited tests by Bushland (1948) showed that a 0.2% toxaphene suspension controlled a hog louse (*Haematopinus adventicus* Neum.) for at least a month. Additional investigations indicated that a 0.5% toxaphene spray would kill all lice present on the animals but should be repeated within 10 to 14 days to eliminate late-hatching lice (Cobbett & Bushland 1956). However, toxaphene is not included in the current recommendations of the Entomology Research Division for hog louse control because of lack of information on the amount stored in the tissues.

In 1957 a cooperative experiment was undertaken at Kerrville, Texas, to study the residues in hog tissues following spray treatments with toxaphene. Eighteen hogs that had not been exposed to chlorinated hydrocarbons were purchased in the vicinity of Kerrville. They were divided into three groups of six each. One group, which served as a control, was sprayed once with water; the second and third groups were sprayed with a 0.5% toxaphene emulsion prepared from a concentrate containing 25% of toxaphene, 65% of xylene, and 10% of Triton X-100. The third group was sprayed again 2 weeks later. The animals were thoroughly wet, and no precautions were taken to keep the spray out of the eyes, nose, or mouth.

Three hogs from each treatment group were sacrificed after 4 weeks and the other three after 6 weeks. Since the end of the 6-week period for the single-treated group coincided with that of the 4-week period for the group treated twice, there were only three slaughter periods. Two control hogs were sacrificed at each period. One hog receiving two sprays farrowed normally after the treatments and was removed from the test.

Samples of omental and renal fat were taken from each slaughtered animal, frozen, packed in dry ice, and shipped to the Hercules Powder Company for chemical analysis.[2] The toxaphene residues were determined by a modification of the total-chlorine combustion method of Hudy & Dunn (1957).

The analytical data are presented in table 1. Each determination is the mean of duplicate analyses using 12 to 18 grams of fat. Fat samples were washed exhaustively with hot water to remove chloride ion. When the entire procedure, including washing, was applied to fat fortified with 5 p.p.m. of toxaphene, recoveries of 91 to 109% (average 96%) were achieved.

Table 1.—Parts per million of toxaphene found in the fat of hogs sprayed with 0.5% toxaphene, calculated from organic-chlorine content and corrected for controls.[a]

WEEKS AFTER TREATMENT	NUMBER OF ANIMALS	OMENTAL FAT	RENAL FAT
	One Spray		
4	3	−0.21	0.81
6	3	−1.42	−0.18
	Two Sprays		
4	3	−0.40	1.14
6	2	−1.93	−0.36
Control	6	4.60	3.96

[a] Negative values indicate less organic chlorine than was found in the control sample.

Table 1 shows that, despite the exhaustive washing, organic chlorine was still present in the control fat samples. No toxaphene was present in the omental fat at either 4 or 6 weeks after one or two sprayings of toxaphene. Toxaphene was present in renal fat 4 weeks after treatment, but not after 6 weeks. The residues were greater in the animals that received two treatments.

On the basis of these results, it appears that meat from toxaphene-treated hogs is safe for human consumption if the animals are slaughtered 6 weeks after spraying once or twice with 0.5% toxaphene.

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[1] Accepted for publication November 19, 1959.

[2] Chemical analyses performed by John J. Ford.

Phenococcus solani Ferris, a Toxicogenic Insect[1]

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Phenococcus solani Ferris has not been recorded from pineapple and records of its occurrence anywhere in Hawaii are very meagre. Infestation developed as a contamination in a *Dysmicoccus neobrevipes* Beardsley[2] colony which was being maintained on *Emilia sonchifolia DC.* Separate colonies of *P. solani* were then developed from single gravid females caged on *Emilia* and *Portulaca oleracea* L. If very small seedlings of either plant species were used, the stems turned black and wilt occurred, but this was apparently a secondary infection by fungi. The symptom was very similar to that of damping-off. When larger plants

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[2] Previously known as the green-spotting strain of *Dysmicoccus brevipes* (CKL).

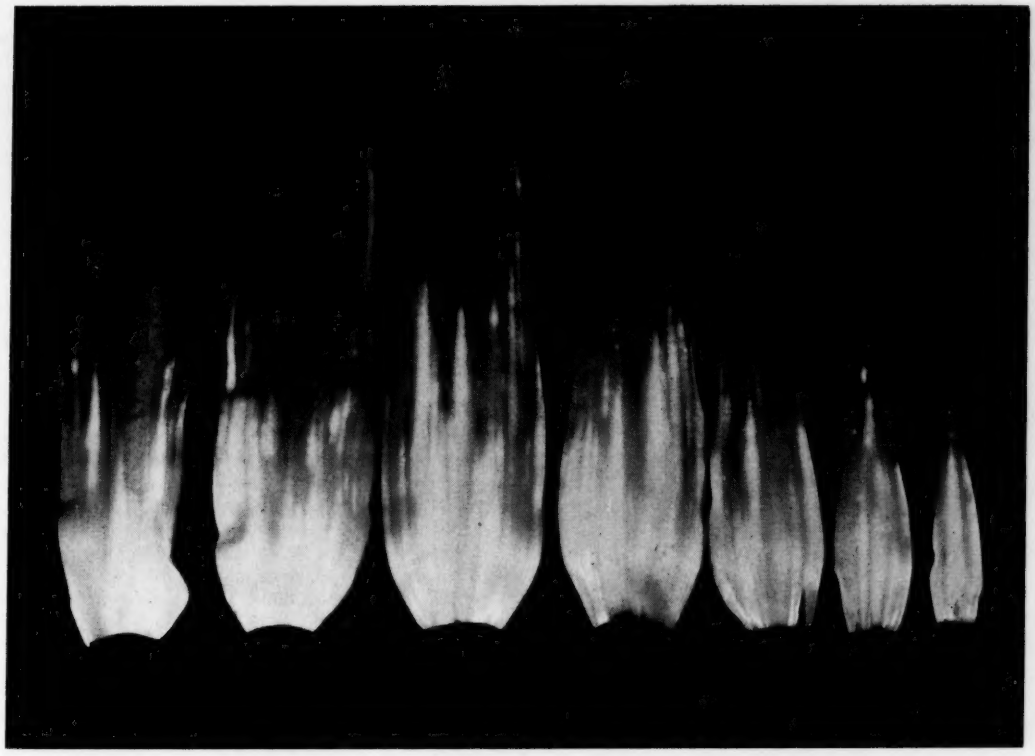

FIG. 1.—Pineapple seedling dissected to show chlorotic spots and streaks resulting from the feeding of *Phenococcus solani*.

of either species were used, large colonies developed before the plants collapsed. It is with the toxic effects of feeding on pineapple plants by the insect that this paper is primarily concerned.

Tissues sought by the insect when feeding on pineapple were the mesophyll cells. Feeding tracks, as revealed by dilute safranin staining, almost invariably ended in mesophyll, occasionally reaching a fibre bundle, but only rarely was a track seen in the vascular region. This was in contrast with those of *D. neobrevipes* which invariably reached the vascular tissue and very frequently touched the fibre bundles. The staining reaction of the track was also different from that of *D. neobrevipes*, which stains clearly and without evidence of cell-wall thickening or diffuse staining on the area contiguous to the track. *P. solani* feeding tracks revealed both these characteristics.

The toxic effects of the insect's feeding were visible on the plant from 7 to 10 days after a single *P. solani* had been allowed to feed. There may be small chlorotic spots visible (fig. 1) but the most frequent and clearly defined symptom was a white streak which broadened as it proceeded in a proximal direction from the feeding point. These streaks appeared on all the fed-on leaves, the youngest showing almost complete involvement. The larger the numbers of insects used, the more rapid was the onset of streaking; 50 mealybugs caused extensive streaking in 4 to 5 days. In the streaked tissue, the chloroplasts were completely destroyed.[3] The development of the streaks in a proximal direction from the feeding point is good evidence for translocation of the toxic entity. Further confirmation was obtained by caging single, or small groups of the insect in felt-plastic cages on the median leaves. Streaks appeared in the center leaves, but only after considerable delay, indicating slower diffusion through mesophyll in the older leaf tissue.

The capacity to induce streaking is inherent in the insect. This was demonstrated by caging gravid females in vials that were covered with tightly drawn cheesecloth and then introduced into a cage containing a seedling pineapple plant. The crawlers can pass through the cloth in a previously unfed state on to the test seedling. Streaking followed this type of infestation in all cases.

Recovery from streaking occurred when the insects were removed. New tissue was streakless and the old streaks sometimes disappeared entirely or became pale green. In these cases, chloroplasts have regenerated.[3]

Streaking was renewed when recovered streaked plants were reinfested with *P. solani* and with the same time elapse before symptom expression. Seedlings infested for the first time were all streaked, but a month later had recovered, the streaks filled in and normal in color. They were reinfested and 5 days later the first new streaks were visible. New streaking was general 8 days after the reinfestation.

Susceptibility to streaking was found without exception. Increased extent and vividness of symptom expression were associated with increased succulence.

[3] I am indebted to Dr. F. Murray Scott for this datum.

Susceptibility of Life Stages of the Mexican Fruit Fly to Fumigation with Ethylene Chlorobromide

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In screening tests by Balock & Lindgren (1951), ethylene chlorobromide appeared promising as a fumigant against eggs and larvae of the oriental fruit fly (*Dacus dorsalis* Hendel). Before more intensive commodity-treatment studies with infested citrus and mangoes at Mexico City were undertaken, it was tested against the naked life stages of the Mexican fruit fly (*Anastrepha ludens* (Loew)).

Fumigations were conducted in a 7.9-cubic-foot lift-up drum

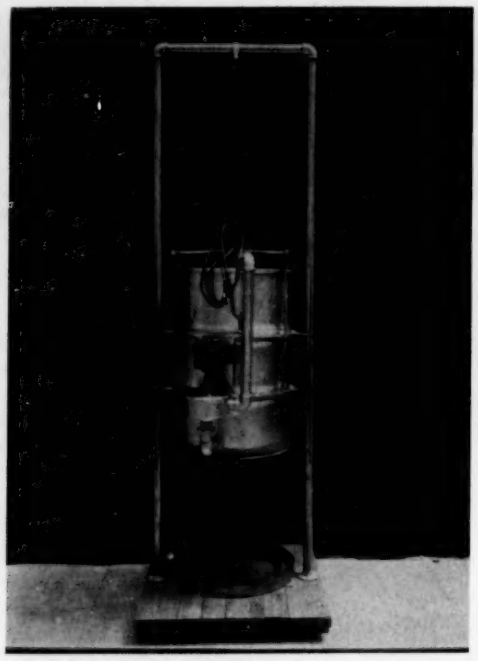

FIG. 1.—Lift-up fumigation drum partially raised to show the water-seal base pan.

chamber provided with a water-seal base pan (fig. 1). The fumigant was introduced with a pipette through a small hole in the top of the drum into a porcelain evaporating dish. This dish was mounted over a 40-watt electric-light bulb, which was used as a heater to vaporize the chemical. The exposure period was 2 hours plus 5 minutes allowed for volatilization of the liquid ethylene chlorobromide. Circulation was not employed. The test insects in their containers were placed on a metal stand which elevated them to the center of the chamber. Laboratory-reared insects were used. The treatment temperature was 73.5° to 78° F. Against each stage from five to seven dosages were tested, in the following ranges (ounces per 1,000 cubic feet):

Eggs	0.25 –3.0
Larvae	0.063–1.5
Pupae	0.5 –8.0
Adults	0.125–1.5

Eggs and larvae were blotted before fumigation to remove surface moisture. These stages could be maintained in a dry condition for several hours without adverse effect.

Eggs about 1 day old were fumigated dry in petri dishes (9 cm.), four lots of about 600 eggs being treated with each dosage. The eggs were then placed on moist white blotting paper in covered petri dishes and held at 78° F. and 90% R. H. Hatch counts were made 7 days later.

Mature larvae were fumigated in large petri dishes (14 cm.), three to five lots of 500 at each dosage. They were then allowed to pupate in a sand-vermiculite mixture with an initial moisture content of 8% by volume. The pupae were held at 78° F. and 70% R. H. Mortality was based on the number of adult flies that eventually emerged.

Pupae 5 to 9 days old were placed on 9-inch squares of fine-mesh screen wire, three or four lots of 1,000 for each dosage. After fumigation they were placed in dry sand-vermiculite and held under conditions of temperature and humidity similar to those for the larvae. Again mortality was based on the number of emerging adult flies.

Adult flies were treated in cylindrical screen-wire cages

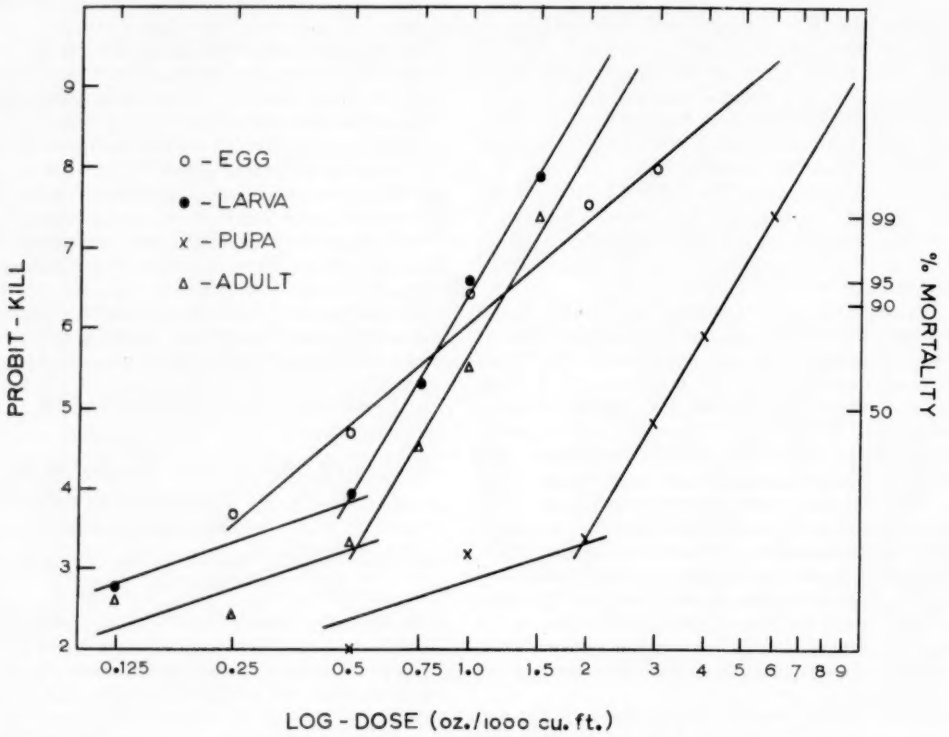

FIG. 2.—Comparison of dosage-mortality relationships for the life stages of the Mexican fruit fly fumigated for 2 hours with ethylene chlorobromide.

(10×6½ inches), 9 to 11 lots of 100 or 200 at each dosage. They were then held in 1 cubic-foot rearing cages with food and water at ±77° F. Humidity was not controlled. No differences were noted between sexes or between sexually mature (15 to 21 days old) and immature (2 to 7 days old) flies; so the data were combined in mortality counts that were made 5 days later.

Dosage-mortality regression lines were prepared for each stage. Mortality in the fumigated lots was corrected for natural mortality in untreated batches. The results are presented in figure 2. The line for eggs is seen to have a very different slope from the others, indicating a difference in rate response. Because of this lack of parallelism, the order of susceptibility at LD-50 was different from that at LD-95. At a 98% to 99% mortality, the level of most practical significance, the order of susceptibility was larva>adult>egg>pupa.

Information on the effect of a fumigant on eggs, larvae, and pupae of this fruit fly has practical value because situations requiring elimination of these stages exist in commercial practice. Adult fruit flies were included primarily for basic information. They are not likely to be present in packages of fresh fruits and vegetables moving in trade, and it is unlikely that a fumigation procedure would be needed for their elimination.

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Natural Factors Control the Pine Tortoise Scale in the Northeast

THOMAS MCINTYRE[1]

The pine tortoise scale, *Toumeyella numismaticum* (P. & M.), ordinarily not destructive in the Northeast, appeared in outbreak proportions in the spring of 1957 in isolated stands of Virginia pine in Maryland, Pennsylvania and West Virginia. Scotch pine trees were attacked to a lesser degree.

In the Lake States this insect attacks jack and Scotch pine and sometimes also red and Austrian pine. It has caused severe damage to jack pine, which appears to be the primary host.

According to Orr (1931), this insect has one generation a year in the Lake States. Studies made during the recent outbreak in the East showed it to have two complete generations a year in Maryland. In the latter area, partially developed females overwintered and matured in the spring; crawlers were abundant from about May 8 to 15; females matured by mid-July; and crawlers again appeared a few days later.

Orr cites six species of Coccinellid predators and a chalcid parasite as important control factors of the scale in the Lake States. Of these the predaceous Coccinellid, *Hyperaspis binotata* (Say), was the most abundant. During the course of the recent outbreak in the East, two species of ladybird beetles, *Hyperaspis binotata* (Say) and *H. signata* (Ol.), were extremely effective predators. Larvae of the scale-eating pyralid moth, *Laetilia coccidivora* (Comst.), also contributed in controlling the scale. Epidemic populations of the scale were almost completely eliminated by these predators in all localities in the Northeast during 1957 where the scale had been reported. *H. binotata*, as in Minnesota, was the primary factor in this reduction. No further outbreaks were reported in 1958.

The results of early control tests with oil sprays in Minnesota were usually overshadowed by the excellent reduction in populations on check areas brought about by natural enemies of the scale. During the spring of 1957, a severe infestation on the Beltsville, Maryland Experimental Forest was treated to prevent injury to an important compartment devoted to forest-management research. The stand was sprayed with 1 pound of malathion in 2 gallons of fuel oil per acre to control the first hatch of crawlers. Excellent control was obtained. However, crawlers that emerged 1 week after treatment were unaffected by the spray. Subsequently these, too, were completely eliminated by predaceous Coccinellid larvae that were under female scales at the time of spraying, and were not killed by the chemical. In a nearby unsprayed area the scales were almost eradicated by predators by late summer.

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[1] Entomologist, Northeastern Forest Experiment Station, Forest Service, U. S. Dept. of Agriculture, Laurel, Maryland.

The Effects on House Flies of Adding Wheat Germ and Corn Oils to Fly Media[1]

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Very little work has been done with the vitamin E (alpha tocopherol) requirements of insect nutrition. Wheat germ oil is a good source of vitamin E and other tocopherol isomers, and may contain up to 550 mg./100 grams, depending on the method of extraction (Lange 1950). House & Barlow (1958) found that the house fly, *Musca domestica* L., reared in synthetic media containing no riboflavin, calcium pantothenate, nicotinic acid or biotin, exhibited significant mortality during the first instar. Significant mortality of the third instar occurred when thiamin was omitted. The emission of choline or pyridoxine did not affect larval survival but led to significant mortality at pupation. These seven vitamins were considered dietary requirements of the larvae.

Brookes & Fraenkel (1958) studied the nutritional requirements of house fly larvae and found that in all experiments the vitamin requirements were met by adding solutions containing nine vitamins: Biotin, choline, folic acid, inositol, nicotinic acid, pantothenic acid, pyridoxine, riboflavin, and thiamin. In these studies a blow fly diet, described by Brust & Fraenkel (1955), proved inadequate for rearing house fly larvae. Carbohydrates, wheat germ oil, and L-cysteine were added but did not prove to be growth accelerators. The addition of dried brewers yeast, however, was considered an improvement to the diet. Oils added to the synthetic media included soybean, wheat germ, cod liver, herring, olive, and butter. Some inhibition in adult size occurred when cod liver oil, herring oil, and butter were added to the media. All the oils reduced the percentage of adults emerging from the media as compared with the check, with the exception of the lower concentration of olive oil, which produced a 95% emergence compared with the 91.4% of the check.

This general lack of knowledge of the influence of vitamin E in insect nutrition and the definite results that have been obtained with mammalian nutrition prompted the undertaking of this preliminary study.

METHODS.—Eight consecutive generations of house flies were reared in Chemical Specialties Manufacturers Association (CSMA) fly media which contained 1% (wet weight) of wheat germ oil,[3] corn oil,[4] and untreated media as a check. Four additional generations were treated in the same manner with 2% of the oils.

The oil was added to the dry media and thoroughly mixed by hand before the aqueous fraction was added. Two battery jars each were treated with wheat germ oil and corn oil and a single

[1] Accepted for publication November 16, 1959.

[2] Assistant Professor and Research Assistant, respectively.

[3] Rex wheat germ oil, Viobin Corp., Monticello, Illinois. Each ounce of oil is the extract from 30 pounds of wheat.

[4] Mazola corn oil, Corn Products Refining Co., Argo, Illinois.

Table 1.—Means of 11 measurements of flies grown on CSMA media.

Observations	8 Generations				4 Generations			
	Check	1% Corn Oil	1% Wheat Germ Oil	Difference Discovered 80% of Time[a]	Check	2% Corn Oil	2% Wheat Germ Oil	Difference Discovered 80% of Time[a]
Average dry weight: males (mg.)	3.46	3.64	3.93	0.62	4.15	4.01	3.91	0.77
Average dry weight: females (mg.)	5.87	6.14	5.67	.99	5.63	5.73	4.76	1.16
Average longevity: males (days)	6.11	8.05	8.16	.78	7.91	7.87	7.97	1.12
Average longevity: females (days)	10.98	11.74	11.60	.97	9.98	10.12	10.38	1.17
Sex ratio (% males)	44.88	45.18	43.81	2.03	41.57	42.73	43.10	5.23
Volume eggs per female (cc.)	.0442	.0494	.0496	.006	.0288	.0219	.0234	.028
Volume eggs/female longevity (cc.)	2.71	2.08	2.30	.46	1.56	1.15	1.48	.46
Hatch (24 hours) (%)	78.18	82.44	83.09	5.54	93.62	92.29	95.62[b]	2.97
No. pupae per ml.	21.27	20.66	20.50	.96	17.52	17.62	16.16[b]	1.28
Average pupal weight (mg.)	24.1	24.9	25.2	10.01	25.5	26.6	27.6	4.41
Emergence (%)	87.93	82.48	83.12	7.33	75.99	79.38	79.74	11.21

[a] See text.
[b] Difference in corn oil and wheat germ oil means significant at 5% level.

jar was left untreated as a check. To each jar was added 0.3 cc. of eggs (approximately 2,000), measured in a graduated centrifuge tube.

Approximately 600 to 700 eggs were placed on a disc of moistened blue blotter paper in a covered petri dish and held 24 hours for hatching. Each disc was then examined under a binocular microscope and the total and unhatched eggs counted.

At the end of 8 days the pupae were separated from the media and placed on a screen tray tilted before an electric fan. The pupae were then measured in a 100-ml. graduated cylinder, weighed, counted and placed in 1-cubic-foot cages containing 6-ounce wax-coated cardboard sundae-cups containing diluted dry skim milk on cellucotton. This diet was changed daily and served both as adult food and oviposition site.

All dead flies were removed daily from each cage, counted, sexed and placed in a container to air dry for 7 to 14 days after the last addition of dead flies. They were then weighed and the average dry weights were calculated.

Oviposition usually began on the fourth day after emergence. Eggs for planting and viability tests were collected from each cage on the second day of oviposition. Consecutive generations were reared in similarly treated media to determine if the oils had any cumulative effects.

In the daily change of the milk cups all eggs were collected, washed with tap water, and measured in a graduated centrifuge tube. A running total of eggs was kept on each cage.

RESULTS.—The means of 11 measurements for each of the 3 media are presented in table 1. The main objective of the experiment was to compare the characteristics of the flies grown on corn oil with those grown on wheat germ oil. Data from these two diets only were used in the statistical analysis.

Because only one jar of check media was planted for each treatment the data from the check treatment did not lend itself to the analysis used. As expected, considerable variation occurred from from generation to generation. However, analysis of the generation differences did not reveal any meaningful biological conclusions. No evidence was observed of any carryover effect of the diet on subsequent generations.

As shown in the table only 2 of the 22 comparisons of corn oil and wheat germ oil were significant at the 5% level. Both of these occurred in the 2% media. The diet effects in these two measurements (per cent hatch, and number of pupae) are not the same in the 2% media and the 1% media. One could logically suppose that the 2 out of 22 significant differences were chance effects. The ingredients of the two oils appear to be neither essential nor valuable to flies when added to CSMA larval media.

An additional column in the table labeled "Difference discovered 80% of time" is a measure of the precision of the test. Since most of the differences were insignificant, the question is posed of how small a difference between corn oil and wheat germ oil could be detected by this test. If a difference as large as the value found in this column of the table had actually existed between the two diets, the test would have divulged it 80% of the time. These values should be of use to future research in this area.

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Residue Studies of Livestock Sprays Containing Sevin[1]

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Sevin® (*N*-methyl-1-naphthyl carbamate) has shown promise against the goat biting louse (*Bovicola caprae* (Gurlt)), the angora goat biting louse (*B. limbatus* (Gerv.)), and the goat sucking louse (*Linognathus stenopsis* (Burm.)) (Moore *et al.* 1959), the winter tick (*Dermacentor albipictus* (Pack.)) (Drummond *et al.* 1959) and horn flies (*Siphona irritans* (L.)) affecting livestock. Tests against horn flies indicated 4 to 6 days' control at 0.5% (Roberts 1959) and more than 2 weeks' at 1.0% (Hays 1959).

In the spring of 1958 workers at the Kerrville, Texas, laboratory showed that when Sevin was fed to dairy animals, it was not secreted in the milk. To determine whether Sevin would be secreted in the milk or stored in the tissues when applied as a water-base spray, additional residue experiments were undertaken.

Two Jersey cows and two Hereford steers were sprayed four

[1] Accepted for publication November 23, 1959. In cooperation with the Union Carbide Chemicals Company.
[2] Entomology Research Division.
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times at 4-day intervals with a 0.5% suspension of Sevin. The 4-day interval was chosen because this was the minimum time that Sevin had been found to protect animals against horn flies.

Milk was taken before treatment, and at various times after each treatment. The milk was weighed, and the amount of butterfat determined. Two 400-ml. aliquots were taken from the milk produced by each cow for chemical analysis. All except the 5-hour sample were taken from the morning milk. Omental fat samples were taken from the Herefords according to the method described by Radeleff (1950) 1 week before treatment, immediately preceding the second treatment, and 5 days after the fourth treatment. The milk and fat samples were shipped to the Beltsville, Maryland, laboratory for analysis.

Milk samples were extracted with a 3:1 mixture of *n*-hexane and methylene chloride. Before extraction the cream was permitted to separate, and the volume of the sample reduced to 150 ml. by siphoning off the milk, since it had been shown that Sevin is found in the cream layer. Three extractions were made, each with 150 ml. of solvent. The combined extracts were dried over anhydrous sodium sulfate and filtered, and the solvent was removed on a steam bath with a 3-ball Snyder column to prevent loss of Sevin. The butterfat was transferred to a separatory funnel with 150 ml. of *n*-hexane and extracted three times with 20-ml. portions of acetonitrile. The acetonitrile extracts were combined, diluted with 200 ml. of water, and extracted with ether. The ether was removed cautiously with a Snyder column, and the residue carried through the procedure developed by Union Carbide Chemicals Company (1958) for raw agricultural commodities.

No determinations for 1-naphthol were made on the milk samples, since it was found that solutions of this compound in methylene chloride were not stable, and the extracts had been held for several weeks before analysis.

Milk samples were analyzed 5 hours and 1 and 2 days after the first spray, 5 hours and 2 days after the second spray, 2 days after the third spray, and 5 hours, 1, 2, and 4 days after the fourth spray. Sevin was not found in any of these samples. Since these were the samples in which Sevin was most likely to appear, the other milk samples were not analyzed.

The fat samples were extracted by maceration in a mortar with anhydrous sodium sulfate followed by grinding with several portions of ether. Only a small amount of connective tissue did not dissolve. The ether was removed and the residue treated in the same manner as the milk samples, except that analyses were made for both Sevin and 1-naphthol.

All the fat samples were analyzed. There were no residues of Sevin or 1-naphthol found in the omental fat samples from the Herefords.

It appears that Sevin can be safely applied to lactating dairy cattle and to beef cattle as a conventional water-base spray for horn fly control without the danger of residues apearing in the milk or tissues.

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A Laboratory Rearing Method for *Lygus hesperus* Knight[1]

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A satisfactory laboratory method for rearing *Lygus hesperus* Knight has been developed. To conduct controlled experiments with insecticides it is necessary to minimize the wide variability encountered in field-collected populations so that only natural or acquired levels of susceptibility are measured. This is best accomplished by using laboratory-reared specimens of uniform age and nutritional background.

The rearing method described here was adapted from one suggested by Menusan (1943) and Sailer (1952) for stink bugs. Fresh green pole-type beans (*Phaseolus vulgaris*) provide food and oviposition sites. Green beans are usually available in grocery stores throughout the year, but a few plants were maintained in a greenhouse during the winter for an emergency supply. To date, insecticide contamination of beans obtained from commercial sources has not been a problem, but such may occur.

Attempts to culture lygus on green pods of cowpea (*Vigna sinensis*) and cotton, alfalfa, cowpea, and bean plants were unsatisfactory. Caged plants presented handling difficulties, and yields of individuals were too low for practical consideration. The culture method of Geering (1953), in which sorghum heads were used as food and oviposition hosts was considered cumbersome and presented problems in availability of host material.

Rearing was conducted in a laboratory maintained at 78° to 80° F. Relative humidity, while uncontrolled, remains near 40% in winter and 60% in summer. Temperature and humidity within the rearing cages has not been measured. Lighting was from overhead fluorescent lamps, regulated with an automatic switch to give 13 light-hours per day.

Parent cultures of lygus were obtained from desired areas by sweeping alfalfa hay fields with an insect net. The lygus bugs were separated from other fauna and trash by releasing the collection in a darkened room and aspirating the adults from a door screen.

Rearing cages were wide-mouth Mason-type jars topped with screen to confine adults and with organdy to prevent escape of nymphs. Adult individuals numbering 60 to 80 were placed in each jar with approximately 2 string beans to every 20 bugs. This ratio of lygus to beans reduced the rate of breakdown and mold of beans while being held for egg hatch. Field-collected adults usually deposited eggs for about 2 weeks. Fresh beans were placed in the jars daily and beans containing eggs were removed to separate jars. Under the laboratory conditions described the eggs hatched 6 to 8 days after deposition. One bean was sufficient for about 100 first-instar nymphs or about 10 fifth-instar or adult bugs. Host material usually lasted about 3 days for numphs during early development and about 2 days during later development.

The time required for nymphal development was about 16 days with developmental time variations up to 4 days. Under the conditions of this rearing method females had a 10- to 13-day preoviposition period. In considering the developmental period of the various lygus bug stages, the total time required to pass through one generation was about 32 to 41 days.

Mortality of bugs during rearing was relatively low. Some individuals were injured by handling or failed to molt properly. These injured or otherwise abnormal individuals were usually attacked by normal adults and nymphs. High mortality occurred during hot summer weather when laboratory temperatures exceeded 95° F. No pathogens were detectable during these occasions and it was assumed that mortality was the result of excessive temperature.

Preliminary bioassay tests with DDT have indicated no sig-

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nificant shifts in dosage-mortality curves in the first 12 laboratory-reared generations of lygus bugs.

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The Malathion Dust-Bath for Control of Five Species of Lice on Chickens[1]

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Common species of lice infesting hens in southern California are the chicken body louse, *Menacanthus stramineus* (Nitz.), the fluff louse, *Goniocotes gallinae* (DeG.), and the shaft louse, *Menopon gallinae* (L.). Occasionally these three species occur on the bird at the same time. Usually, however, the infestation is of one species. Less common are the wing louse, *Lipeurus caponis* (L.), and the chicken head louse, *Cuclotogaster heterographus* (Nitz.). The large chicken louse, *Goniodes gigas* (Tasch.) and the brown chicken louse, *G. dissimilis* Denny, were rarely found in this area.

The success of dust-bath boxes (Rodriguez & Riehl 1957) charged with 4% malathion dust to control the chicken body louse of hens on litter indicated that further investigation of the method for the control of other lice on chickens would be of economic interest.

MATERIALS AND METHODS.—The experiments reported here were conducted on six poultry establishments with floor-management operations and designated here as A, B, C, D, E, and F. For those with litter on the floor, dust-bath boxes were used, and for those with range-type pens, the natural dusting wallows in the soil were treated (Rodriguez & Riehl 1957). One pound of 4% malathion dust per wallow or per dust-bath box (3′ long by 2′ wide by 1′ deep) was used for each 100 hens. All treatments were replicated at least three times and on each ranch one pen was left as the untreated check.

On ranches A, B, and C litter was used in the floor-management operation. One dust-bath box per 100 birds was used for the application of malathion; boxes were placed over favorite wallows and the charge of malathion dust added. At each of ranches A and B there were 300 to 400 hens per pen and the infestation consisted of mixed populations of the chicken body louse, the fluff louse, and the shaft louse. At ranch C, there were 500 hens per pen infested with the fluff louse.

The birds at ranches D, E, and F were kept in range-type pens. The charge of malathion dust was put into the wallows. On ranch D there were 25 laying hens per pen infested with the shaft louse. On ranches E and F the flocks were composed of Bantam chickens. There were 100 birds per pen at ranch E infested with the wing louse. On ranch F there were 30 birds per pen infested with the head louse. The pens with 25 and 30 birds received ¼ and ⅓ pounds respectively of 4% malathion dust put into one wallow only per pen.

The procedures used to measure louse infestations were the same for the six ranches. The birds were rated prior to treatment and counts of lice were made thereafter at 7-day intervals. Ten per cent of the hens in the large pens at ranches A, B, C, and E and 50% of the birds in the small flocks at ranches D and F were caught at random and examined in the vent and fluff areas, on the side, under the wings, and on the neck, head, and breast. If lice were present, the species were noted; records were kept of the percentage of hens infested rather than the number of individuals per species of lice per bird.

Table 1.—Average percentages of chickens infested with lice at intervals after treatment with 1 pound of 4% malathion dust per 100 birds applied in dust-bath boxes or natural wallows.

	Species of Lice on the Hens at Each Ranch						
	Litter on Floor			Range-Type			
Days	A Body Fluff Shaft	B Body Fluff Shaft	C Fluff	D Shaft	E Wing	F Head	Untreated Check
	Percentage of Hens Infested						
0	100	100	100	100	100	100	100
7	0	30	0	0	3	0	100
14	0	3	0	0	0	0	100
28	0	0	0	0	0	0	100
56	0	0	0	0	0	0	100
70	0	0	0	0	0	0[a]	100
84	0	0[a]	0	0	0[a]	—	100
108	0[a]	—	0	0[a]	—	—	100

[a] Observations discontinued because pens culled.

DISCUSSION OF RESULTS.—Results of the trials are shown in table 1. The pretreatment counts on all the ranches showed that 100% of the birds were heavily infested with the species of chicken lice indicated. At ranches A, B, and C the hens were free of lice in from 7 to 14 days after the dust-bath boxes were put into the pens and remained free of lice from 84 to 108 days after treatment. On ranches D, E, and F the birds were free of lice 7 days after the charge of malathion dust was put into the natural wallows and they remained free of lice from 84 to 100 days thereafter.

These data show that use of 4% malathion dust in the dust-bath box in pens with litter or in natural wallows in range-type pens is an efficient method of controlling each of five species of lice occurring singly or in mixed populations on chickens in floor-management poultry ranches.

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Malathion Dust for Chicken Mite Control[1]

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In southern California the chicken mite, *Dermanyssus gallinae* (DeG.), is a common and serious pest of chickens reared on litter in floor-management houses or in community-type cages with a wire floor above the ground supported by a wooden framework (Dougherty & Belton 1929) in which the mites find suitable hiding places for existence. Usually the chicken mites emerge from their shelters during the night to feed on the roosting hens. Although in this area the mite can appear at any time of the year, extra vigilance should be practiced in the spring and early summer. The chicken mite causes serious stress to the laying hens and a heavy infestation of mites may decrease egg production (Bishopp & Wood 1917).

Generally, in the past, acaricides were applied to the roost in

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aqueous sprays or paints (Hall & Wehr 1953). Both techniques required mixing and considerable time and labor for application, particularly applied as a paint to the floor joists of community cages. The application of aqueous mixtures by spray or paint often resulted in wet spots in the droppings or litter, thereby increasing the potential for flies and diseases. Thefore, it seemed desirable to explore the use of dust as a method of chicken mite control.

Good control of the chicken mite in wire cages with no wooden parts demonstrated by Rodriguez & Riehl (1958) with 4% malathion dust made it the material of choice for trials in floor-management houses or in community cages with wooden construction.

MATERIALS AND METHODS.—Four ranches in the Riverside area were selected for the tests. Ranches A and B used floor-management houses with 300 to 600 hens per house. Ranch C used community cages (floor dimensions 6 by 8 feet) with 50 to 70 pullets per cage. Ranch D used both types of pens with 16 hens per community cage (4 by 5 feet) or 250 to 300 hens in floor-management houses.

Estimates of mite infestations were made at each of 10 sites selected at random at each pen unit at each count interval. At each count site the debris that could be dislodged readily from cracks or crevices was pushed or scraped out of an area of approximately 1 square inch. In all instances prior to treatment mites were so numerous that ratings (heavy, medium, or light) were more practical than actual counts.

Treatment consisted of 4% malathion dust applied with a modified bellows-type hand duster equipped with an extension tube 3 feet long on the nozzle. Two pounds of 4% malathion dust per 36 lineal feet were applied to all wooden surfaces of roosts, floor joists, feed troughs and nest boxes; feed and eggs were removed before application. Point blank force was used to drive the dust into cracks, and underneath dried droppings and debris. Particular attention was given to all possible hiding places that could harbor mites.

The number of pen units treated with 4% malathion dust at each of the ranches was 8 houses at ranch A, 10 houses at ranch B, 32 cages at ranch C, 25 cages and 7 houses at ranch D. At each ranch two pen units were left untreated as checks. Following application of the dust treatments, observations of mite populations were made each week.

DISCUSSION OF RESULTS.—The results of the study are shown in table 1; the data on mite populations are summarized by months. Prior to treatment all the pen units were heavily infested with all stages of the chicken mite. No live mites were found at any of the observation sites in the treated pens at 7 days or thereafter for at least 150 days at ranches A, B, and D and for 90 days at ranch C, when the pullets were culled and taken out of the community cages and moved into floor-management houses. The untreated pens were maintained for 90 days at all four ranches.

The data show that 4% malathion dust applied with a bellows-type hand duster is an efficient method of treatment for the chicken mite. Complete control was obtained for at least 5 months. Time and effort for dusting were less than that required for the liquid applications used previously, particularly for community-type cages; a single pen unit can be treated in a few minutes. Wet spots in the litter and manure are avoided.

At the present tine a concentration of 4% malathion is the lowest readily available commercially in dust formulations. For application with a hand duster a volume of 2 pounds of dust is just adequate for practical minimal coverage of the surface represented in 36 lineal feet.

Table 1.—Chicken mite control in hen houses and community cages with 2 pounds of 4% malathion dust per 36 lineal feet of the wooden surfaces of roosts, feed troughs, nest boxes and exposed floor joists applied with a modified bellows-type hand duster.

DAYS AFTER TREATMENT[a]	AVERAGE RATING OF INFESTATION AT POULTRY RANCHES					
		Floor-Management Houses			Community Cages	
	Untreated	A	B	D	C	D
7	Heavy	0	0	0	0	0
14	Heavy	0	0	0	0	0
30	Heavy	0	0	0	0	0
60	Heavy	0	0	0	0	0
90	Heavy	0	0	0	0	0
150	[b]	0	0	0	[c]	0

[a] All ranches were heavily infested before treatment.
[b] Discontinued by control treatment application made at 90 days.
[c] Pullets culled and moved to floor-management houses at 90 days.

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A Technique Useful in Comparing Powder-Post Beetle Populations in the Substructures of Buildings[1]

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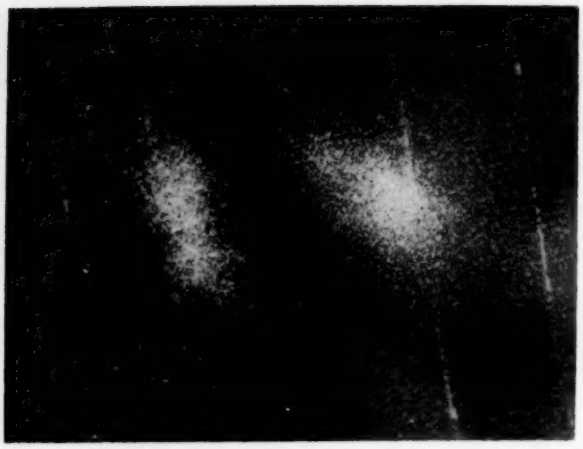
Lack of a satisfactory method of comparing powder-post bettle populations in the subflooring and/or joists of buildings has hindered field research with this group of insects. The following technique enabled a comparison of beetle activity in several areas under a house to be made.

Three houses of similar construction, with crawl areas 3 to 4 ft. high, and built on adjoining lots near Raleigh, North Carolina, were located. Each contained an active beetle infestation spread over at least 800 sq. ft. of exposed wood surface. Larvae and adults collected from the wood were identified by T. J. Spilman and Jerome G. Rozen, Entomology Research Division, U. S. Department of Agriculture, as *Xyletinus peltatus* Harris. For simplicity these houses were designated as A, B and C.

Eight rectangular areas under each house, containing approximately 100 sq. ft. of infested exposed wood surface per area, were outline with a wax crayon line. Strips of felt paper, 3×9 ft., were placed under the various areas and the number of piles of powder which fell on the papers were recorded (fig. 1).

Observations were taken at 1- and 6-day intervals, for it was not known how many piles would be collected, and it was thought that the counting of them on the felt papers at the end of a 6-day period might be an impossibility because the entire surfaces of the felt papers would be covered with powder. Because of this, 1-day observations were also recorded, with 6-day and 1-day

[1] Accepted for publication December 1, 1959.
[2] Now with the Wilson Exterminating Company, Winston-Salem, N. C.



intervals following each other. Subsequent records indicated that observations could be made every 7 days; therefore, weekly observations were initiated. The powder piles present on the felt papers were removed after they were counted.

The number of piles gave an estimate of *X. peltatus* activity in the joists and subflooring. When beetle activity was high a large number of piles were formed and when activity was low few were formed.

FIG. 1.—Area on a felt paper showing two piles of powder which fell from sub-flooring and joist infested with *Xyletinus peltatus*.

BOOK REVIEWS

MOSQUITOES OF MEDICAL IMPORTANCE, by RICHARD H. FOOTE AND DAVID R. COOK. 158 pages, 68 figs., 357 ref. Superintendent of Documents, U. S. Government Printing Office, Washington 25, D. C. $2.50 (Buckram).

The purpose of this book is to make possible rapid identification of those species of mosquitoes that are of medical importance in any given region of the world. The means for so doing is presented as a series of pictorial keys to larvae and adult females, which sort out the important species but eliminate those not known to be vectors of malaria, yellow fever, dengue, and the encephalitides. Also included are brief discussions on biology and medical importance of the species. The keys are easy to use. Each distinguishing character, which is clearly illustrated, is followed by a bracket containing the species possessing this character and these species in turn are divided by brackets set apart by characters which also are illustrated.

With this kind of guide, even poorly trained entomological technicians should experience little difficulty in assigning names to those specimens which they have not discarded. This book should be of use to health officers, engineers, and other people who may have limited entomological training, whose duties require rapid identification of problem mosquito species. It will also be of use to the professional entomologist, who may not be a taxonomist or who is a specialist in other groups, but who may be a member of an epidemiological or control team, and whose duties require him to identify the medically important species.

However, one must recognize that this kind of guide has its limitations. Most professional entomologists, especially those specializing in mosquitoes, would be dissatisfied with keys which permit identification of only a few species. It is probable that, with the exclusion of so many species which prohibits comparisons, many specimens may be wrongly assigned to the species illustrated. For example, the rather difficult *Arribalzagaia* group contains species that may be run out to *A. punctimacula* in the key to species of northwest South America; the superficial resemblance of *A. apicimacula* and *neomaculipennis* to the illustration of *A. punctimacula* may be more impressive than the small characters separating these species, without greater emphasis through word description or illustrations.

Nevertheless, the book attains its objective of providing a rapid guide to important species. The illustrations are well drawn and clearly reproduced. The notes on distribution, biology, and disease transmission combine with keys to give a concise summary of the medically important mosquitoes of the world.

LLOYD E. ROZEBOOM

A SYNOPTIC CATALOG OF THE MOSQUITOES OF THE WORLD (DIPTERA, CULICIDAE). By ALAN STONE, KENNETH L. KNIGHT AND HELLE STARCKE. Published by the Entomological Society of America, Washington, D. C. Thomas Say Foundation (Publication). Vol. 6, 358 pp. 1959. $7.25.

Owing largely to the importance of mosquitoes as disease vectors, world catalogs of this group of insects are of interest and importance not only to taxonomists but to investigators in many fields of pure and applied biology. The present catalog fills admirably the need for a guide to all the known mosquito taxa and indirectly to the vast literature that has accumulated since the appearance 27 years ago of Edwards' masterly review and catalog of this family (Wytsman's General Insectorum, fasc. 194, 1932).

The scope of the present catalog is more restricted than that of Edwards since only the subfamily Culicinae, raised to family rank, is covered and no systematic diagnoses, discussions or keys are given. This catalog is synoptic only in the sense that it does not cite all the literature pertaining to every taxon and includes references to the systematic status of taxa only if a change has been proposed since 1932. Because of these features the new catalog does not replace but rather supplements and corrects that of Edwards.

A tremendous amount of work was done in the preparation of this catalog. The authors checked the original descriptions or proposals of every included nominal taxon (339 in the generic group and 4067 in the specific group, and 2 generic and 35 specific *nomina nuda*), examined or obtained data on all the existing type specimens, and reviewed the type fixations for all the generic group taxa. Through the initiative of the two senior authors a number of long-standing controversial nomenclatural problems have been finally resolved by action of the International Commission on Zoological Nomenclature (type species of *Anopheles* and *Toxorhynchites* (=*Megarhinus*); suppression of

Taeniorhynchus and validation of *Mansonia*). The user of this catalog will find a number of nomenclatural changes which are necessary because of homonymy or priority; the majority of these have been explained in recent publications by the two senior authors and other specialists but a few appear here for the first time. A number of unresolved nomenclatural problems are also indicated in the catalog. In all nomenclatural matters except in the treatment of infrasubspecific forms the authors have adhered to the new International Rules of Zoological Nomenclature (in preparation). Although the advisability of some of the changes may be questioned by some specialists (for example, resurrection of *Cellia* for *Myzomyia*), this catalog provides for the first time a sound basis for nomenclatural stability in the mosquitoes and it is hoped that it will be so used by everyone even in the case of *Culex* (*C.*) *quinquefasciatus* (=*fatigans*).

The arrangement and presentation of the data are excellent and are fully explained in the introduction to the catalog. A very useful feature is the inclusion of general references to the anatomy, bionomics, systematics and faunal works for the family and important systematic papers for all the generic group taxa. The generic group taxa have full data on the type species fixation. The information given for the specific group taxa is more extensive than in Edwards and includes type locality, type location, selected references to descriptions and figures of all stages, bionomics and changes in systematic status since 1932 in addition to the usual complete citation of the original description. Edwards' groups, subgroups and series are eliminated and all specific group forms are arranged directly under the appropriate genera or subgenera.

It is perhaps unreasonable to expect more from a catalog than is given in the present one, but one wishes that the authors had included a brief statement of larval habitat wherever known since this information is fully as important as the type locality and distribution. It may have been possible also, without much additional work, to indicate by an appropriate symbol whether or not the described sexes and immature stages have been definitely associated by individual or progeny rearings. As in all other synoptic catalogs the distribution data are not supported in many cases by literature references and it is therefore impossible to verify them directly from the catalog. Since some records were missed, some erroneous ones included, and no distinction was made between introductions and indigenous distributions, the data presented are not entirely reliable.

The catalog is unusually free of clerical or typographical errors but in the publication of a work of this type and magnitude it is inevitable that a few escape detection in spite of all precautions. I have noted several minor ones in checking over species represented in the South Pacific and these range from omission of a specific name (*Culex nigriceps* Buxton, 1927), unexplained transfer (*tonnoiri* to *Culiseta* from *Climacura*), incorrect distributions (several) to typographical and other errors in literature citations.

It cannot be said that advances in classification have been comparable to the increase in knowledge of species. That nearly all the species discovered in the last 27 years have found convenient resting places in the classification of Edwards is in a large part a tribute to his taxonomic acumen and the soundness of his system but also indicates a lack of broad revisionary studies since his day and an unwillingness to depart from a well-established tradition. In the present catalog the tribes of Edwards have been elevated to subfamily rank and have become the Anophelinae, Toxorhynchitinae and Culicinae. In the Culicinae the five generic groups of Edwards have been discarded and only the Sabethini and Culicini are recognized as tribes. The same number of genera, 31, are used but 3 of the genera recognized by Edwards have been synonymized (*Goeldia*, *Sabethoides*, *Dendromyia*), *Phomomyia* has been resurrected and *Ayurakitia* and *Udaya* (both questionably distinct from *Aedes*) have been added. The number of subgenera has considerably increased, to some extent by elevation of Edwards' species groups, so that the total number of valid generic and subgeneric taxa has increased from 89 to 111 (not 110 as stated in the introduction and exclusive of one fossil genus which is not listed in the catalog).

The authors have modified Edwards' arrangement of genera in their tribe Culicini to some extent in line with recent speculations as to possible affinities but in my opinion their arrangement is not an improvement nor is the lumping of 4 of Edwards' generic groups into this one tribe. The lack of revisionary work has prevented the authors from preparing diagnoses for supraspecific categories and from a rearrangement of intrageneric groups. It is clear that the present classification is a stopgap extension of that of Edwards and that an improvement will come only when all the groups are thoroughly restudied on as broad a basis as possible. The present catalog should serve as an impetus towards this objective but it is unfortunate, in my opinion, that it deals only with the Culicidae in the restricted sense and disregards the Dixinae and Chaoborinae, much neglected groups that are undoubtedly closely related to the true mosquitoes and appear to show many clues to an understanding of the morphology and evolution of the Culicinae.

JOHN N. BELKIN,
University of California

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OBITUARY

Otto Herman Swezey
1869-1959

On November 3, 1959, Dr. Otto Herman Swezey died at the age of 90 in a sanitarium near San Jose, California; thus ending a long career as one of Hawaii's most distinguished and deeply respected scientists. Forty-eight of his active years were spent in Hawaii and his achievements in the service of the Hawaiian sugar planters and to the community as a whole have been outstanding.

Dr. Swezey was born June 7, 1869, on a backwoods farm 6 miles east of Rockford, Illinois. His ancestors came to Salem, Massachusetts, from England in 1632. Many of the family have since become prominent in the fields of music and medicine. His early school days were spent in what was then the Guilford Center School House, described by him as a one-room wooden building in an area miles removed from a church, store, railroad station or even a post office. When about 16 or 18 years of age his teacher, who was a university graduate, influenced him to enter a university and he began his collegiate training at Lake Forest College, Illinois, where he received his Bachelor of Arts degree in 1896. In 1897 he was granted a Master of Arts degree at Northwestern University, Illinois, where he had a Fellowship during 1896–1898. He served at Northwestern as biology instructor during 1898–1902. He then obtained a Fellowship at Ohio State University as a research student in entomology, where he studied during 1902–1903 under Professor Herbert Osborn, who greatly stimulated his interest in a study of the Hemiptera-Heteroptera of the State. He was also employed through 1903 and part of 1904 as Assistant Orchard Inspector for the Ohio State Department of Agriculture.

From some brief autobiographical notes Dr. Swezey prepared a few years ago, we learn that he first became interested in insects through reading a small book entitled "Insect Lives or Born in Prison." Amongst insects illustrated in this book was the beautiful Cecropia moth, *Samia cecropia*, and its cocoon. He was then 6 or 8 years of age. During the winter he found such a cocoon on an apple tree in the garden and brought it into the house, where it was hung on the wall. One evening the following March a fine Cecropia adult emerged. This was the start in a life-time interest in rearing Lepidoptera. His first experience in economic entomology began with efforts to reduce damage to potato foliage in the garden by the Colorado potato beetle. He shook the insects into a pan held below and killed them with hot water or kerosene and later sprinkled the infested rows with paris green suspended in a bucket of water.

By 1904, Dr. Swezey had published several papers on economic leafhoppers and their parasites in Ohio. At that time the sugarcane leafhopper, *Perkinsiella saccharicida* Kirk. threatened the very existence of the sugar industry in Hawaii. Owing to the excellence of his published papers on leafhoppers and through the suggestion of Dr. L. O. Howard, Dr. Swezey was employed by the Hawaiian sugar planters as Assistant Entomologist at the HSPA Experiment Station about June, 1904. He married Mary H. Walsh on July 20, 1904, and they came to Hawaii on August 12. It is of record that Dr. Swezey became the first American entomologist to be employed by a private organization. In 1915 he was promoted to the position of Entomologist at the Experiment Station to be held until his retirement in 1933, when he became Consulting Entomolgist. Though retired, he continued his entomological pursuits in Hawaii for more than 18 years. His long and faithful service to the Hawaiian sugar industry was outstanding. He played a major role in the successful biological control of insects attacking sugar cane in Hawaii and the sugar industry must always remain indebted to him.

In 1907 he was appointed Honorary Curator of insect collections at the Bernice P. Bishop Museum, a service he generously assumed in his spare time for the next 12 years. His help was sorely needed and appreciated by the Museum and he had a great deal to do with the organization, preservation and expansion of the insect collections of that institution. In 1920 the Museum appointed him Consulting Entomologist and in later years Honorary Consultant.

Otto Herman Swezey
1869–1959

Dr. Swezey was granted an Honorary Degree of Doctor of Science in 1944 by the University of Hawaii, an honor to which few are more deserving. He was a member of the American Association for the Advancement of Science, the American Assocation of Economic Entomologists, the Entomological Society of America, the Hawaiian Entomological Society, the Hawaiian Botanical Society and the Hawaiian Academy of Science. He was President of the Hawaiian Entomological Society four times and Editor of its Proceedings for 39 years. As Editor he performed an extraordinary service in the editing of the first 11 volumes. This exacting and trying work was a duty he cheerfully assumed. He set a standard of excellence and precision that has served as a model for ensuing editors.

Under the auspices of the Hawaiian Sugar Planters' Association, Dr. and Mrs. Swezey spent 7 months in 1936 conducting an entomological survey of the island of Guam. They were assisted part time by Dr. Robert L. Usinger and Mr. E. H. Bryan, Jr. This resulted in the collection of many hundreds of insect species accompanied by essential notations with reference to host plants, et cetera. This material, plus some collected by

D. T. Fullaway in 1911, formed the basis for two volumes published by the Bishop Museum under the title "Insects of Guam" and which now serve as a major reference work on the insects of that Island.

Dr. Swezey visited Samoa in 1923 for 4 weeks to observe and report on the economic insects of the area. Much information of definite reference value was obtained. Again in 1940 he spent 13 weeks in Samoa with Dr. E. C. Zimmerman making a general entomological survey. This resulted in the assembly of the largest collection of Samoan insects ever made and it will be many years before the material can be studied and reported upon in detail by specialists.

Dr. Swezey left hundreds of records covering his observations on the habits and identity of Hawaiian insects. He has published more than 230 papers or bulletins, mostly with reference to Hawaiian insects. Their reference value will gain rather than lose in usefulness. He was always more interested in assembling facts regarding the biologies of insects rather than in the description of new species. Though most of the new species discovered by him were described by other workers, he has described 107 of Lepidoptera. We note that 78 insects have been named after him.

Dr. Swezey's greatest contributions to entomology deal with the Hawaiian insects; but especially the Lepidoptera. Dr. E. C. Zimmerman's "Insects of Hawaii" is dedicated to Dr. Swezey and Dr. C. Montague Cooke. In Volume 7 of the series, he pays a moving tribute to Dr. Swezey in which he states, in part, that "Much of what I have recorded herein regarding the habits and early stages of the Hawaiian Lepidoptera has come from the researches of Dr. Swezey. This book would be a mere shadow of itself if it were not for the observations made by him over a period of nearly 50 years. No other person or combination of persons, has contributed so much to our knowledge of the biologies of the Lepidoptera of Hawaii. Dr. Swezey was absolutely devoted to his studies, and he assembled an enormous mass of records on the biologies of Hawaiian insects. We are indebted to him for the bulk of our knowledge on the habits, host plants, parasites and early stages of Hawaiian Lepidoptera."

Dr. Swezey was especially interested in the endemic insect fauna of the Hawaiian forests. For many years almost all of his holidays were spent in collecting in the mountain forests and few persons have ever explored these regions as extensively as he did. In his host plant interests he became an authority on the endemic Hawaiian flora as well as the insects. In the course of these studies he gradually assembled an impressive mass of carefully recorded notes. He had long planned to publish the results of this work. During his last few years in Hawaii his colleagues urged him to do so. As a crowning culmination and climax to his long career as an entomologist, he finally put the finishing touches to his manuscript on October 15, 1952, just a few hours before his steamer bore him and his good wife permanently from Hawaii. This manuscript was published by the Bernice P. Bishop Museum August 2, 1954, in collaboration with the Hawaiian Botanical Society and the Hawaiian Entomological Society, under the title "Forest Entomology in Hawaii," and appeared as Special Publication 44. Its 266 pages are packed with concise and reliable information on the insect faunas of the components of the Hawaiian Forests.

Dr. Swezey was greatly revered by all who knew him. His wide interest and his enthusiasm in studying the Hawaiian biota was stimulating to his colleagues. He was tolerant of all conditions and people that confronted him and was prone to overlook or excuse the faults or mistakes of others, rather than criticize. And finally though rich in achievements and honors in his chosen field of science, he always remained a modest and unassuming man.

He is survived by his wife and one son, Joseph A. Swezey, and two grandchildren.

C. E. PEMBERTON

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